# NOVEL NIOSOMAL DRUG DELIVERY SYSTEM OF ETOPOSIDE FOR TARGETING DIFFERENT TYPES OF CANCER

**A Dissertation Submitted to**

**The Tamil Nadu Dr. M.G.R. Medical University**

**Chennai - 600 032**

**In partial fulfillment for the award of Degree of**

**MASTER OF PHARMACY**

**(Pharmaceutics)**

**Submitted by**

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**(Accredited by "NAAC" with a CGPA of 2.74 on a four point scale at 'B' grade)**

**MELMARUVATHUR - 603 319**

**MAY 2012**

# CERTIFICATE

This is to certify that the dissertation entitled **"NOVEL NIOSOMAL DRUG DELIVERY SYSTEM OF ETOPOSIDE FOR TARGETING DIFFERENT TYPES OF CANCER"** Submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the award of the Degree of the Master of Pharmacy was carried out by **S. GAYATHRI (Register No. 26106001)** in the Department of Pharmaceutics under my direct guidance and supervision during the academic year 2011-2012.

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# CERTIFICATE

This is to certify that the dissertation entitled **"NOVEL NIOSOMAL DRUG DELIVERY SYSTEM OF ETOPOSIDE FOR TARGETING DIFFERENT TYPES OF CANCER"** is the bonafide research work carried out by **S.GAYATHRI (Register No. 26106001)** in the Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under the guidance of **Mr. T. AYYAPPAN, M. Pharm.,** Assistant Professor, Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, during the academic year 2011-2012.

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# My Heartfelt Dedication

# To

# Almighty God

# &

# My Beloved Parents

# ACKNOWLEDGEMENT

First and foremost, I wish to express my deep sense of gratitude to his Holiness **ARULTHIRU AMMA,** President, ACMEC Trust, Melmaruvathur for their ever growing Blessings in each step of the study.

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**S. GAYATHRI**

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# ABBREVIATIONS

| | |
|---|---|
| % | ---- Percentage |
| °C | ---- Degree Celsius |
| µg | ---- Microgram |
| µg/mL | ---- Microgram per milliliter |
| BBB | ---- Blood brain barrier |
| cm | ---- Centimeter |
| CNS | ---- Central nervous system |
| DNA | ---- Deoxy ribonucleic acid |
| DSC | ---- Differential Scanning Calorimetry |
| edn | ---- Edition |
| EE | ---- Entrapment efficiency |
| eg | ---- Example |
| Eq | ---- Equation |
| ETP | ---- Etoposide |
| Fig | ---- Figure |
| FTIR | ---- Fourier Transform Infra Red Spectroscopy |
| gm | ---- Grams |
| $g/cm^3$ | ---- Gram per cubic centimeter |
| h | ---- Hours |
| HCl | ---- Hydrochloric acid |
| ICH | ---- International Conference on Harmonization |

| | |
|---|---|
| I P | ---- Indian Pharmacopoeia |
| LE | ---- Loading efficiency |
| ml | ---- Milliliter |
| mg | ---- Milligram |
| mg/mL | ---- Milligram per milliliter |
| N | ---- Normality |
| NDDS | ---- Novel drug delivery system |
| nm | ---- Nanometer |
| No. | ---- Number |
| PBS | ---- Phosphate buffer saline |
| pH | ---- Negative logarithm of hydrogen ion |
| RES | ---- Reticulo endothelial system |
| rpm | ---- Revolutions per Minute |
| SD | ---- Standard Deviation |
| SEM | ---- Scanning Electron Microscope |
| S.No. | ---- Serial Number |
| t | ---- Time |
| TODDS | ---- Target oriented drug delivery system |
| UV | ---- Ultra Violet |
| w/v | ---- weight in volume |
| w/w | ----weight in weight |
| λmax | ---- Absorption maximum |

# INTRODUCTION

# 1. INTRODUCTION

In the past few decades, considerable attention has been focused on the development of new drug delivery system (NDDS). Carrier-mediated drug delivery has emerged as a powerful methodology for the treatment of various pathologies. The therapeutic index of traditional and novel drugs is enhanced via the increase of specificity due to targeting of drugs to a particular tissue, cell or intracellular compartment, the control over release kinetics, the protection of the active agent or a combination of the above.

Targeted drug delivery is the most important goal of pharmaceutical research and development. In principle, drug targeting can be achieved by physical or biological that result in high concentrations of the pharmacologically active agent at the pathophysiologically relevant site. The advantages to the targeted release system is the reduction in the frequency of the dosages taken by the patient, having a more uniform effect of the drug, reduction of drug side effects, and reduced fluctuation in circulating drug levels.

The result of the targeting would be a significant reduction in drug toxicity, reduction of the drug dose, and increased treatment efficacy. TODDS have been developed to optimize regenerative techniques. The system is based on a method that delivers a certain amount of a therapeutic agent for a prolonged period of time to a targeted diseased area within the body. This helps maintain the required plasma and tissue drug levels in the body. Therefore, avoiding any damage to the healthy tissue via the drug.

## 1.1. Novel Drug Delivery Systems:

(*Shobha Rani R.H.,2008; Robinson Joseph R, Lee V.H.L., 2009)*

For past decades, the acute or chronic disease mostly commonly treated by the delivery of drug to patients by using various pharmaceutical formulation such as capsules, pills, creams, tablets, suppositories, ointments, liquids, aerosols, and injections. But to maintain the drug concentration within the therapeutically effective range for treatment, it is necessary to take the dosage forms several times a day, resulting in significant fluctuations of drug levels in the body. To minimize this fluctuation, novel drug delivery systems have been developed, which include niosomes, liposomes, nanoparticles, microspheres, micro-emulsions, impalatable pumps and magnetic microcapsules.

The NDDS should ideally fulfill two prerequisites: firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of treatment. Secondly, it should channel the active entity to the site of action. Conventional dosage forms including prolonged release dosage forms are able to meet none of these. At present no available drug delivery systems behave ideally, but sincere attempts have been made to achieve the above mentioned prerequisites them through various novel approaches in drug delivery.

Recently, different carrier systems and technologies have been extensively studied with the aim of controlling the drug release and improving the efficacy and selectivity of formulation. Nowadays, vesicles as a carrier system have become the vehicle of choice in drug delivery and lipid vesicles were found to be of value in immunology, membrane biology, diagnostic technique and most recently in genetic engineering.

## 1.2. Targeted Drug Delivery System:

*(Banker S.G and Rhodes C.T, 2008; Vyas SP, Khar R.K., 2002)*

By definition targeted drug delivery is a strategy aiming at the delivery of a compound to a particular tissue in the body. Ideally, drug targeting would provide a high local concentration of drug at the site of disease and a concentration below levels of toxicity in healthy tissues.

The idea of targeting to a specific site in the body was first introduced almost a century ago by Ehrlich. However only in recent years has the field emerged as an important area of research. For the new drugs, and for some conventional drugs (e.g., antineoplastic agents) that have narrow therapeutic windows and require localization to a particular site in the body, it is essential that they be delivered to their target sites intact, in adequate concentration, and in an efficient, safe, convenient, and cost-effective manner.

When the drug is administered by the parenteral route, the problems associated with the gastrointestinal tract are avoided. However, deactivation and metabolism of the drug and dose-related toxicity is frequently observed. There are many diseases such as cancer, rheumatoid arthritis and certain bacterial and fungal parasitic infection that are inadequately accessible to drugs. The treatment of these diseases often entails high frequent dosing of drugs, which can lead to toxic magnifications, inappropriate pharmacodisposition and untoward metabolism. Targeted drug delivery system designed for cancer predominantly avoids “normal” tissue and targets cancer cell specifically.

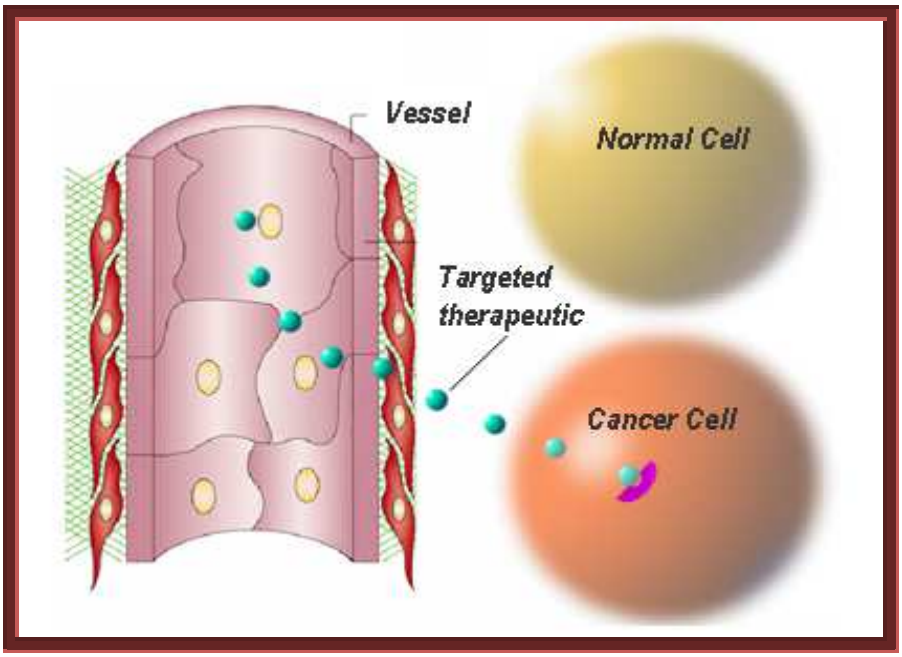



**Fig. 1.1:** Diagrammatic representation of challenges facing targeted therapeutics

### 1.2.1. Classification of drug targeting:

Targeting with drug- carrier system can be divided into three types, they are:

- Passive targeting
- Active targeting (ligand mediated targeting and physical targeting)
- Physical targeting

**Passive targeting** relies on the natural distribution pattern of the drug- drug-carrier system. For example, particle with diameter of 5 µm or smaller are readily removed from the blood by macrophages of the RES when administered systematically. Mechanical filtration of particulate carriers larger than 5-7 µm by capillary blockage can also be exploited to target drugs to the lungs by the venous supply and to other organs through the appropriate arterial supply.

**Active targeting** employs a deliberately modified drug- drug-carrier molecule capable of recognizing and interacting with a specific cell, tissue, or organ in the body. Modification of the carrier system may include change in the molecular size, alteration of the surface properties, incorporation of antigen-specific anti-bodies, or attachment of cell receptor- specific ligands.

**Physical targeting** refers to delivery systems that release a drug only when exposed to a specific microenvironment such as a change in ph or temperature, or the use of an external magnetic field.

### 1.2.2. Requirements of Targeted Drug Delivery Systems:

- Internalize and intracellularly traffic to site of intended action
- Avoid “normal” tissue
- Remain intact until reaching its intended site of action
- The delivery system should be biologically inert
- It must be non-immunogenic, physically and chemically stable in vivo and in vitro.
- The carrier must be biodegradable or readily eliminated without any problem
- The delivery system must be reproducible cost-effective and simple.

### 1.2.3. Systems for targeted drug delivery:

*(Banker S.G and Rhodes C.T, 2008; Robinson Joseph R, Lee V.H.L., 2009; Khar R.K and Vyas S.P., 2008)*

Drug- carrier- delivery system employs biologically inert macromolecules to direct drug to its target site in the body. These are divided into two types: **particulate**

and **soluble macromolecular**. Depending on the carrier system, the drug can be either molecularly entrapped within the carrier matrix or covalently linked to the carrier molecules.

❖ **Particulate drug delivery systems:**

The concept of using particles to deliver drug to selected sites in the body originated from their use in medicine and in the investigation of the RES (liver, spleen, and bone marrow), and lymph nodes, gastrointestinal examination, and so on. Particle ranging in sizes from 20 to 300µm have been proposed for drug targeting. Because of the small size of the particles, particulate drug-delivery system can be introduced directly into the central nervous injection or delivered to a given body compartment.

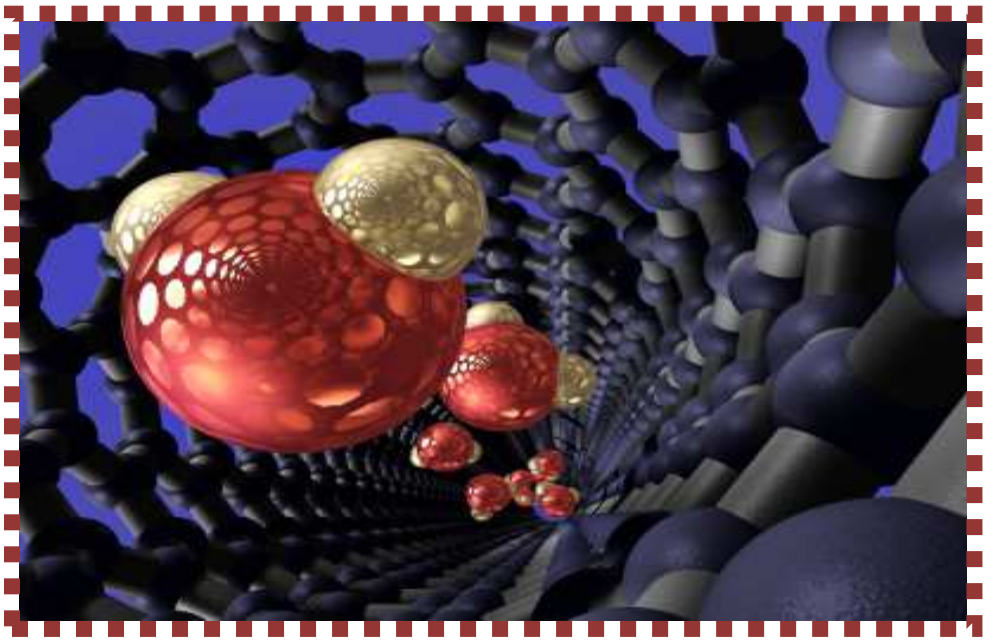


**Fig. 1.2:** Highly targeted particulate drug carrier system

➢ The various particulate drug-carrier system that has been investigated can be grouped as following.

**Table 1.1.** Various carrier and particulate delivery system

| S. No | Carrier System | Size Range | Features | Method Of Preparation |
|---|---|---|---|---|
| 1 | Solid lipid nanoparticle | 50-1000nm | Submicron colloidal carriers containing solid hydrophobic core having a monolayer of phospholipids coating. | .High-pressure homogenization<br>.Microemulsion formation<br>.Precipitation<br>.As lipid nanopellets |
| 2 | Nanotubes and Nanowires | - | self-assembling sheet of atoms arranged in the form of tubes and thread-like structures of nanoscale range | Surface functionalization |
| 4 | Liposomes | 25nm-100µm | microscopic vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments | .Mechanical dispersion<br>.Solvent dispersion<br>.Detergent removal |
| 5 | Niosomes | 10 to 1000 nm | non-ionic surfactant vesicles are bilayered structures | -Hand shaking<br>-Reverse phase evaporation<br>-Ether injection method<br>-Trans membrane |
| 6 | Lipidmicrotubules/ microcylinders | <1µm | self organizing system in which surfactants crystallize into tightly packed bilayers that spontaneously form cylinders | Self emulsification |
| 7 | Lipospheres | 0.2-100µm | water dispersible solidmicro particles composed of solid hydrophobic fat core stabilized by a monolayer of phospholipids molecules embedded in a microparticle surface | .Melt method<br>.Multiple microemulsion<br>.Cosolvent method<br>-Preincorporation into lipophilic carrier |

Particulate drug-delivery system depends on the size, shape, charge and surface hydrophobicity of the particles. After intravenous administration, particle larger then 7µm are normally mechanically filtered by the smallest capillaries of the lungs (particle of 15 µm have been homogeneously distributed throughout the lungs, whereas particle of 137 µm exhibited a more peripheral distribution and particle smaller than 7µm in diameter (between 2 and 7 µm) may pass the smallest in the capillary network of the liver and spleen. For organs that bear solid tumors, this approach may lead to localization in the tumor cells.

**For parenteral administration**, drug delivery systems to be safe and effective they require the following properties: they should be small in particle size (nm range), biocompatible, bio-degradable, devoid of immunogenicity, have high drug-loading capacity, exhibit long blood circulation , extravasate into the required pathological sites and be able to release the drug contents at the target site for therapeutic effect.

**Particulate delivery system** like liposomes, micelles and polymeric nanoparticles has been extensively studied with a great deal of success and provide most of the properties desired in delivery systems for parenteral administration. Because of their small size and ability to functionalize their surfaces with various polymers or ligands, these particles can be used for target specific delivery. Various other constructs or assemblies exist which can serve the same function of targeted delivery include drug- polymer conjugates, cyclodextrins, niosomes, solid lipid particles, lipoproteins, microemulsions, dendrimers, metal nanoparticles, protein cages, polyplexes, and cochle.

**Table 1.2:** Main applications of parenteral particulate drug delivery system

| S.no | Drug delivery technology | Main applications |
|---|---|---|
| 1 | Liposome | Passive tumour targeting<br>Vaccine adjuvants<br>Passive targeting to lung endothelium in gene delivery<br>Targeting to regional lymph nodes<br>Targeting to cell surface ligands in various organs/areas<br>Sustained release depot at point of injection |
| 2 | Niosome | Passive tumour targeting<br>Vaccine adjuvants<br>Sustained release depot at point of injection |
| 3 | Nanoparticle | Passive tumour targeting<br>Vaccine adjuvants |
| 4 | Microparticle | Sustained release depot at point of injection<br>Vaccine adjuvants |
| 5 | Cyclodextrin | Lipophilic drug solubilisation for Parenteral use |
| 6 | Emulsion | Lipophilic drug administration vehicles<br>Targeting to cell surface antigens |
| 7 | Prodrug<br>i) ADEPT<br>ii) Polymer drug conjugates | Active tumour targeting<br>Polymer drug conjugates |
| 8 | Polymeric micelles | Active tumour targeting |

### 1.2.4. Different Approaches to Drug Targeting:

*(Gupta Manish and Sharma Vimukutha, 2011)*

- **Ligand identification and tumor targeting:**

The identification of suitable ligands is essential for the development of efficient targeting strategies. Besides antibodies (e.g. single-chain Fv fragments) natural receptor-binding molecules and synthetic peptides can be used as ligands. Such new or improved ligands can be obtained by combinatorial and evolutionary approaches, e.g. in combination with phage display technology. Several phage display ligand libraries have been developed in our group and applied for the isolation of specific ligands. For instance, a fully synthetic antibody library was generated, which can be employed for the isolation of human scFv fragments against virtually any antigen.

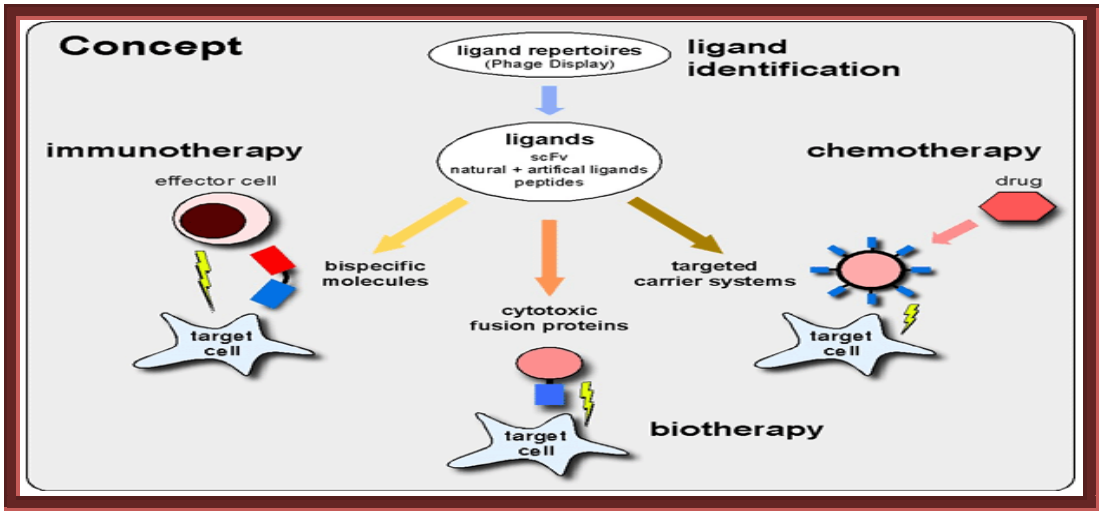



**Fig. 1.3:** Schematic representation of ligand identification & targeting

- **Bispecific antibodies for effector cell retargeting:**

The immune system is composed of an arsenal of different and potent effector cells, e.g. cytotoxic T-lymphocytes or NK cells. Recognition and elimination of

malignant cells by these effector cells is, however, often not very efficient. Bispecific antibodies are capable to redirect effector cells and to trigger killing of target cells, thus improving effector cell cytotoxicity. The recombinant bispecific molecules can be easily generated and produced by genetic engineering.

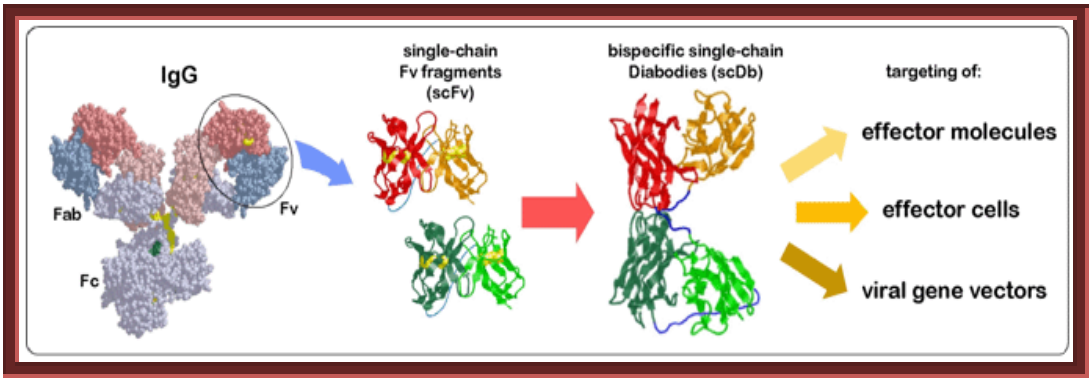



**Fig. 1.4:** Effector cell retargeting

- **Brain targeted drug delivery system:**

The brain is a delicate organ, and evolution built very efficient ways to protect it. The delivery of drugs to central nervous system (CNS) is a challenge in the treatment of neurological disorders. Drugs may be administered directly into the CNS or administered systematically (e.g., by intravenous injection) for targeted action in the CNS. The major challenge to CNS drug delivery is the blood-brain barrier (BBB), which limits the access of drugs to the brain substance. Advances in understanding of the cell biology of the BBB have opened new avenues and possibilities for improved drug delivery to the CNS.

A range of strategies that have been used for manipulating the blood-brain barrier for drug delivery to the brain include osmotic and chemical opening of the blood-brain barrier as well as the use of transport/carrier systems. Other strategies for drug delivery to the brain involve bypassing the BBB.

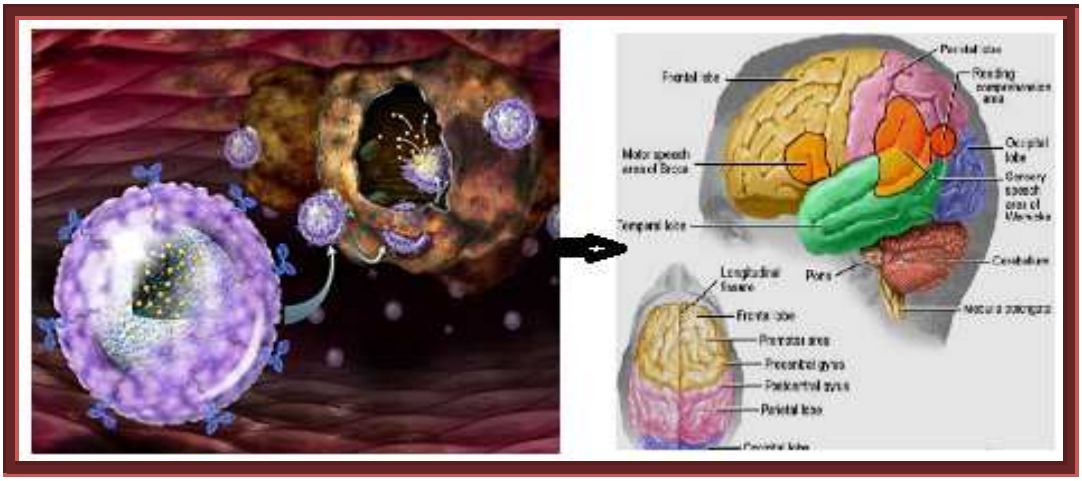


**Fig. 1.5:** Carrier system to access the relevant target site within the CNS.

Various pharmacological agents have been used to open the BBB and direct invasive methods can introduce therapeutic agents into the brain substance. It is important to consider not only the net delivery of the agent to the CNS, but also the ability of the agent to access the relevant target site within the CNS. Various routes of administration as well as conjugations of drugs, e.g., with liposomes and nanoparticles, are considered.

- **Aptamers for Targeted Drug Delivery:**

Aptamers are a class of therapeutic oligonucleotides that form specific three-dimensional structures that are dictated by their sequences. They are typically generated by an iterative screening process of complex nucleic acid libraries employing a process termed Systemic Evolution of Ligands by Exponential Enrichment (SELEX).

Therefore, relatively few aptamers have been generated that bind cell surface receptors. With cell-based selection, a specific protein target is not always chosen, but selection is performed against a target cell type with the goal of letting the aptamer

choose the target. All cell surface proteins cycle intracellularly to some extent, and many surface receptors are actively internalized in response to ligand binding.

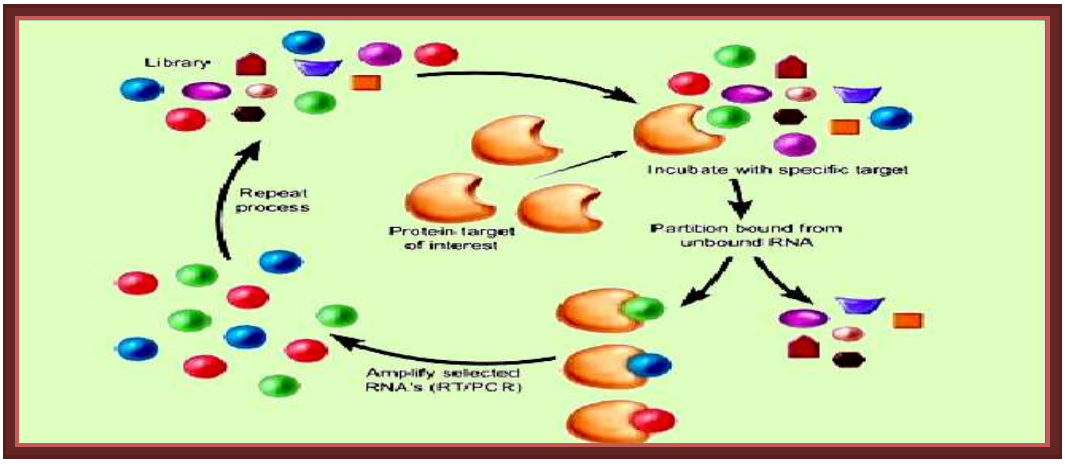



**Fig. 1.6:** Aptamers for targeted drug delivery

- **Carrier systems for targeted drug delivery:**

Many chemotherapeutics are characterized by a limited therapeutic efficacy, often due to severe side effects at higher doses and rapid elimination from the body due to their small size. Encapsulation of small therapeutic molecules into nanoparticular carrier systems, such as liposomes or polymers, can improve the pharmacokinetic and pharmacodynamic properties and protects the active compound from degradation.

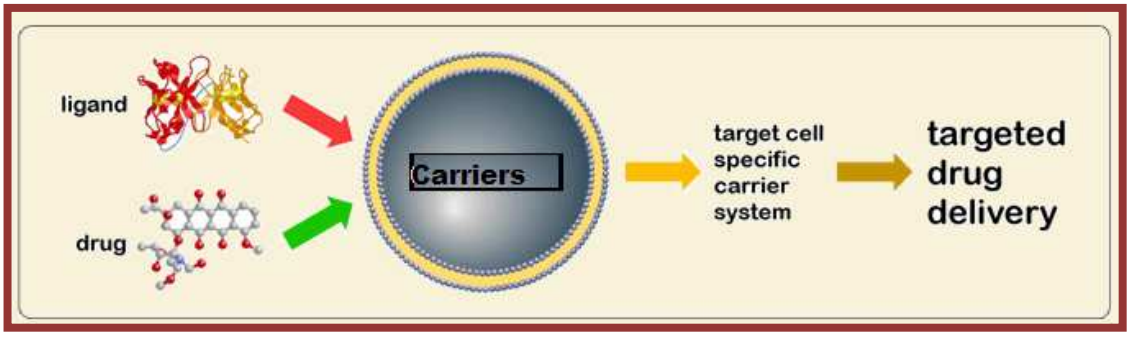



**Fig. 1.7:** Carrier systems for targeted drug delivery

### 1.3. Vesicular system-Carrier for Drug Delivery*:* *(Raju Jukanti, et al., 2010)*

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayer formed, when certain amphiphillic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphillic building blocks. Biologic origin of these vesicles was first reported in 1965 by Bingham, and was given the name Bingham bodies. Drug carrier can be engineered to slowly degrade, react to stimuli and be site-specific.

The ultimate aim is to control degradation of drug and loss, prevention of harmful side effects and increase the availability of the drug at the disease site. Encapsulation of a drug in vesicular structures can be predicted to prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved. Lipid vesicles are one type of many experimental models of biomembranes which evolved successfully, as vehicles for controlled delivery. For the treatment of intracellular infections, conventional chemotherapy is not effective, due to limited permeation of drugs into cells. This can overcome by the use of Vesicular drug delivery systems.

#### 1.3.1. Need for Vesicular drug delivery system:

- Prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved due to the delivery of drug directly to the site of infection.
- Improves the bioavailability especially in the case of poorly soluble drugs.
- Both hydrophilic and lipophilic drugs can be incorporated.
- Delays elimination of rapidly metabolizable drugs and thus function as sustained release systems.

At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery. Recently different carrier systems and technologies have been extensively studied with the aim of controlling the drug release and improving the efficacy and selectivity of formulation. Now a days vesicle as a carrier system have become the vehicle of choice in drug delivery and lipid vesicles were found to be of value in immunology, membrane biology and diagnostic technique and most recently in genetic engineering.

### 1.3.1. Different novel approaches by vesicular system: *(Ravi Kumar, et al., 2011)*

Vesicular delivery system provides an efficient method for delivery to the site of infection, leading to reduce of drug toxicity with no adverse effects. Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both by hydrophilic and liophilic drugs. Different novel approaches used for delivering the drugs by vesicular system include liposomes, niosomes. sphinosomes, transferosomes and pharmacosomes.

- **Liposomes:**

The liposomes have emerged as most practically useful carriers for in-vivo drug delivery as majority of reports has concentrated on the use of phospholipid vesicles or liposomes as potential drug carrier systems. Liposomes are potential carrier for controlled drug release of tumours therapeutic agents and antibody for gene therapy. Liposomes are formed when thin lipid films or lipid cakes are hydrated and stacks of liquid crystalline bilayers become fluid and swell.

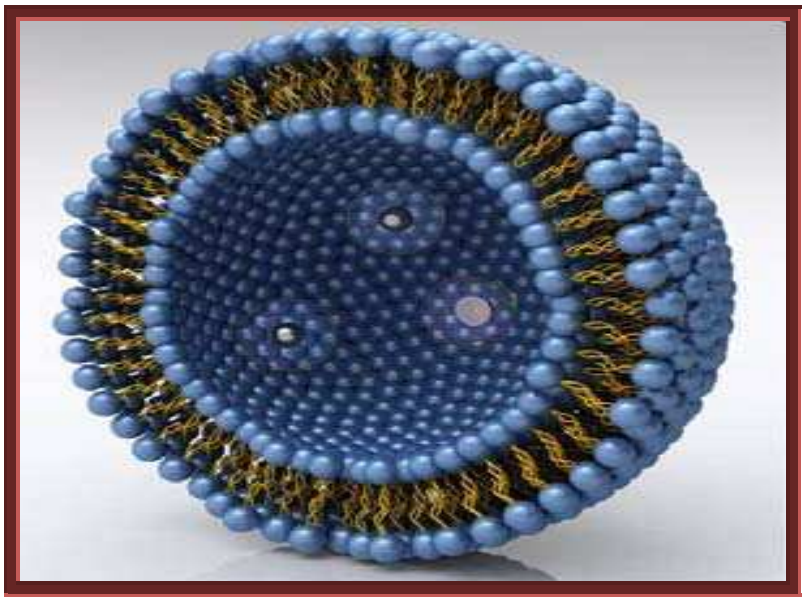


**Fig. 1.8:** Model of a unilamellar liposome consisting of one double lipid bilayer.

- **Niosomes:**

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle. The vesicle is composed of a bilayer of non-ionic surface active agents and hence the name niosomes. The niosomes are very small, and microscopic in size. Their size lies in the nanometric scale. Although structurally similar to liposomes, they offer several advantages over them.

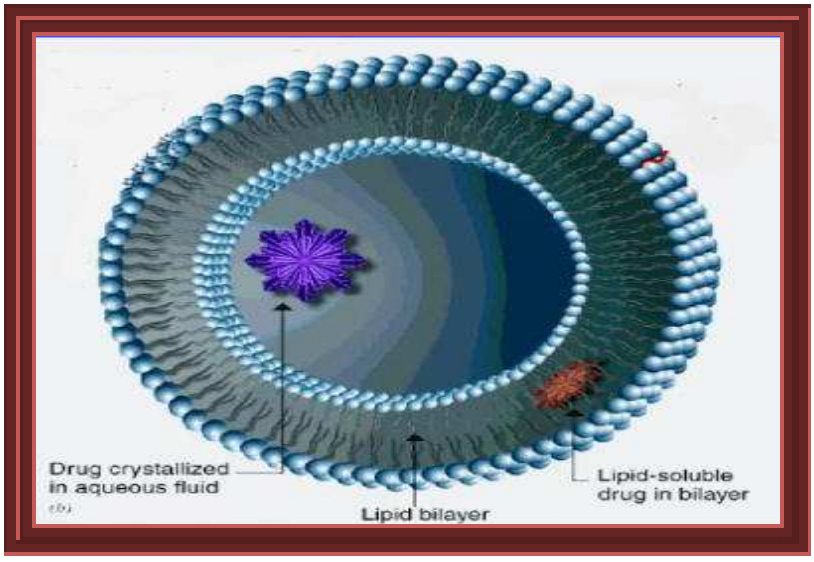



**Fig. 1.9:** Diagrammatic representation of Niosomes

- **Sphinosomes:**

The sphingolipid (contains ether or amide linkage instead of ester linkage) are been nowadays used for the preparation of stable liposomes known as sphingosomes. Sphingosome may be defined as "concentric, bilayered vesicle in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphingolipid.

Sphingosome are administered in many ways these include parentral route of administration such as intravenous, intramuscular, subcutaneous, and intra-arterial. Generally it will be administered intravenous or some cases by inhalation. Often it will be administered into a large central vein, such as the superior vena cava and inferior vena cava to allow highly concentrated solution to be administered into large volume and flow vessels.

- **Pharmacosomes:**

Pharmacosomes bearing unique advantages over liposome and niosome vesicles, have come up as potential alternative to conventional vesicles.

Depending upon the chemical structure of the drug–lipid complex they may exist as ultrafine vesicular, micellar, or hexagonal aggregates. As the system is formed by linking a drug (pharmakon) to a carrier (soma), they are termed as pharmacosomes. They are an effective tool to achieve desired therapeutic goals such as drug targeting and controlled release.

- **Transferosomes:**

Transferosomes was introduced for the effective transdermal delivery of number of low and high molecular weight drugs. Transfersomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that

differ in their bilayers properties. It consist of both hydrophilic and hydrophobic properties, high deformablity gives better penetration of intact vesicles. These vesicular transfersomes are several orders of magnitudes more elastic than the standard liposomes and thus well suited for the skin penetration.

Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum. Transferosome based formulations of local anesthetics- lidocaine and tetracaine showed permeation equivalent to subcutaneous injections. Anti cancer drugs like methotrexate were tried for transdermal delivery using transferosome technology. This provided a new approach for treatment especially of skin cancer.

### 1.3.2. Emerging Vesicular Drug Delivery System: *(Ravi Kumar, et al., 2011)*

- **Aquasomes:** Three layered self assembly compositions with ceramics carbon nanocrystalline particulate core coated with glassy cellobiose Specific Targeting, molecular shielding.
- **Cryptosmes:** Lipid vesicles with a surface coat and of suitable polyoxoyethylene derivative of phosphotidyl ethanolamine. Ligand mediated drug targeting
- **Discomes:** Niosomes solublized with non ionic surfactant solutions (polyoxyethylene cetyl ether class) Ligand mediated drug targeting
- **Emulsomes:** Nanosize Lipid particles (bioadhesives nanoemulsion) consisted of microscopic lipid assembly with a polar core. Parenteral delivery of poorly water soluble drugs.
- **Enzymosomes:** Liposomal constructs engineered to provide a mini

bioenvironmental in which enzymes are covalently immobilized or coupled to the surface of liposomes. Targeted delivery to tumor cell

➢ **Ethosomes:** Lipid "Soft malleable vesicles" embodying a permeation enhancer and composed of phospholipid, ethanol and water. Targeted delivery to deep skin layer

➢ **Genosomes:** Artificial macromolecular complexes for functional gene transfer .Cationic lipids are most suitable because they posess high biodegradability and stability in the blood stream. Cell specific gene transfer.

➢ **Photosomes:** Photolysase encapsulated in liposomes, which release the content photo-triggered charges in membrane permeability characteristics. Photodynamic Therapy

➢ **Vesosomes:** Nested bilayer compartment in vitro via the interdigested bilayer phase formed by adding ethanol to a variety of saturated phospholipids. Multiple compartment of the vesosomes gives better protection to the interior contents in serum.

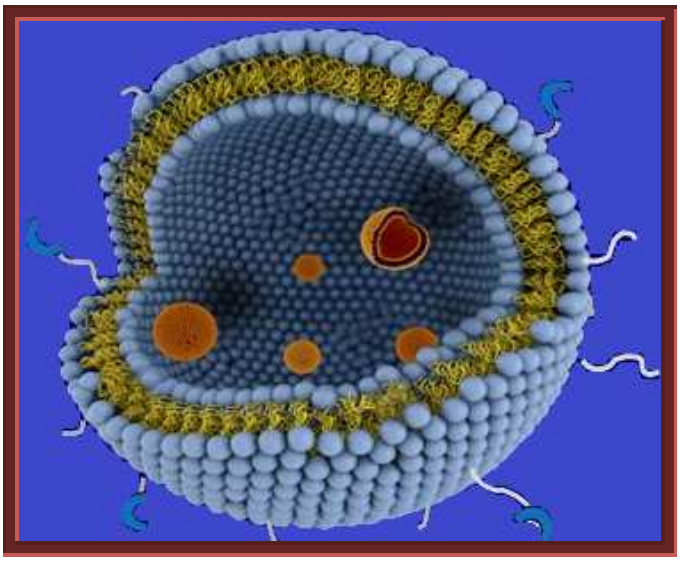


**Fig. 1.10:** Multi compartment structure of vesosomes

➢ **Proteosomes:** High molecular weight multi-submit enzyme complexes with catalytic activity, which is specifically due to the assembly pattern of enzymes. Better catalytic activity turnover than non associated enzymes.

### 1.4. Niosomes:

*(Jaydeep D Yadav, et al., 2011; Rajesh Z. Mujoriya, et al., 2011; Shobha Rani R.H.,2008)*

Niosomes represent a promising drug delivery module. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. Hence they represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multi environmental structure. Niosomes are thoughts to be better candidate's drug delivery as compared to liposomes due to various factors like cost, stability etc.

#### 1.4.1. Historical aspect:

Niosomes were first reported in the seventies as a feature of the cosmetic industry by Vanlerberghe et al, Handjani-vila et al., Van Abbe explained that the non – inonic surfactants are preferred because the irritation power of surfactants decreases in the following order: cationic > anionic > ampholytic > non-ionic.

At present no available drug delivery system achieves the site specific delivery with controlled release kinetics of drug in predictable manner.

Paul Ehrlich, in 1909, initiated the era of development for targeted delivery when he envisaged a drug delivery mechanism that would target directly to diseased cell. Since then, number of carriers was utilized to carry drug at the target

organ/tissue, which include immunoglobulins, serum proteins, synthetic polymers, liposomes, microspheres, erythrocytes, niosomes etc.

Among different carriers liposomes and niosomes are well documented drug delivery. Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media.

### 1.4.2. Description:

Structurally, niosomes are similar to liposomes. Both are made up of bilayer, which is made up of non-ionic surfactant in the case of niosomes and phospholipids in case of liposomes. Both hydrophilic and hydrophobic drugs can be incorporated into niosomes. The niosomes are ampiphillic in nature, which allows entrapment of hydrophilic drug in the core cavity and hydrophobic drugs in the non-polar region present within the bilayer. The structure of niosomes is given in Fig 1.11.

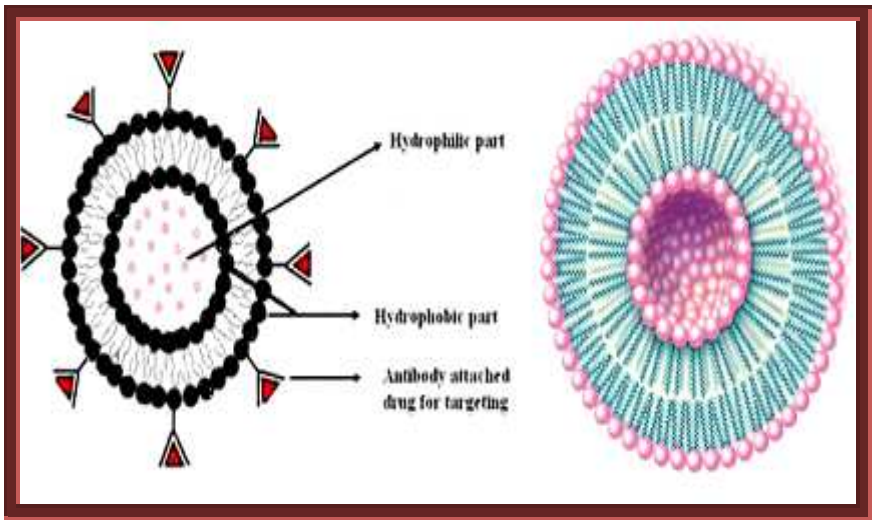



**Fig. 1.11**: Structure of niosomes

### 1.4.3. Advantages of niosomes:

- They entrap solute in a manner analogous to liposomes.

- They are osmotically active and stable.
- Handling and storage of surfactants requires no special conditions.
- The surfactants are biodegradable, biocompatible, and nonimmunogenic
- They possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with a wide range of solubilities.
- They exhibit flexibility in their structural characteristics (composition, fluidity, and size) and can be designed according to desired application.
- They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.
- They allow their surface for attachment of hydrophilic group and can incorporate hydrophilic moieties in bilayer to bring about changes in their in vivo behavior.
- They improve the therapeutic performance of the drug molecules by delaying the clearance from the circulation, protecting the drug from biological environment, and restricting effects to target cells.
- Niosomal dispersion in an aqueous phase can be emulsified in a nonaqueous phase to regulate the delivery rate of drug and administer normal vesicle in external nonaqueous phase.

### 1.4.4. Types of niosomes:

- **Bola Surfactant Containing Niosomes:**

Bola surfactant containing niosomes are the surfactants that are made of omega-hexadecyl- bis- (1-aza-18 crown-6) (bola surfactant): span-80/cholesterol in 2: 3: 1 molar ratio.

- **Proniosomes:**

Proniosomes are the niosomal formulation containing carrier and surfactant, which requires to be hydrated before being used. The hydration results in the formation of aqueous niosome dispersion. Proniosomes decreases the aggregation, leaking and fusion problem associated with niosomal formulation.

- **Aspasomes:**

Combination of acorbyl palmitate, cholesterol and highly charged lipid diacetyl phosphate leads to the formation of vesicles called aspasomes. Aspasomes are first hydrated with water/aqueous solution and then sonicated to obtain the niosomes. Aspasomes can be used to increase the transdermal permeation of drugs. Aspasomes have also been used to decrease disorder caused by reactive oxygen species as it has inherent antioxidant property.

### 1.4.5. Types of niosomal system:

*(Surender Verma, et al., 2010)*

❖ **Small unilamellar vesicles:** (SUV, size 0.025-0.05 μm) are commonly produced by sonication, and French Press procedures. Ultrasonic electrocapillary emulsification or solvent dilution techniques can be used to prepare SUVs.

❖ **Multilamellar vesicles:** (MLV, size >0.05 μm) exhibit increased-trapped volume and equilibrium solute distribution, and require hand-shaking method. They show variations in lipid compositions.

❖ **Large unilamellar vesicles:** (LUV, size >0.10 μm), the injections of lipids solubilised in an organic solvent into an aqueous buffer, can result in spontaneous formation of LUV.

### 1.4.6. Factors affecting formation of niosomes:

*(Dubey subodh, et al., 2010)*

❖ **Nature of encapsulated drug:**

The physico-chemical properties of encapsulated drug influence charge and rigidity of the niosome bilayer. The drug interacts with surfactant head groups and develops the charge that creates mutual repulsion between surfactant bilayers and hence increases vesicle size. The hydrophilic lipophilic balance of the drug affects degree of entrapment.

**Table 1.3:** Effect of nature of drug

| S.no | Nature of drug | Leakage from vesicle | Entrapment | Stability |
|---|---|---|---|---|
| **1** | Hydrophobic drug | Decreased | High | Increased |
| **2** | Hydrophilic drug | Increased | Low | Decreased |
| **3** | Amphiphilic drug | Decreased | High | - |
| **4** | Macromolecule | Decreased | - | Increased |

❖ **Amount and type of surfactant:**

The mean size of niosomes increases proportionally with increase in the HLB of surfactants like Span 85 (HLB 1.8) to Span 20 (HLB 8.6) because the surface free energy decreases with an increase in hydrophobicity of surfactant. The bilayers of the vesicles are either in the so-called liquid state or in gel state, depending on the temperature, the type of lipid or surfactant and the presence of other components such

as cholesterol. In the gel state, alkyl chains are present in a well-ordered structure, and in the liquid state, the structure of the bilayers is more disordered.

❖ **Cholesterol content and charge:**

Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency. An increase in cholesterol content of the bilayers resulted in a decrease in the release rate of encapsulated material and therefore an increase of the rigidity of the bilayers obtained. Presence of charge tends to increase the interlamellar distance between successive bilayers in multilamellar vesicle structure and leads to greater overall entrapped volume. In general, the action of cholesterol is two folds; on one hand, cholesterol increases the chain order of liquid-state bilayers and on the other, cholesterol decreases the chain order of gel state bilayers. At a high cholesterol concentration, the gel state is transformed to a liquid-ordered phase.

❖ **Temperature of hydration:**

Hydration temperature influences the shape and size of the noisome. The hydrating temperatures used to make niosomes should usually be above the gel to liquid phase transition temperature of the system.

❖ **Membrane composition:**

The stable niosomes can be prepared with addition of different additives along with surfactants and drugs. Niosomes formed have a number of morphologies and their permeability and stability properties can be altered by manipulating membrane characteristics by different additives.

### 1.4.7. Similarities between Liposome and Niosome:

♦ The liposomes and niosomes are functionally same.

- Both can be used in targeted and sustained drug delivery system.
- Property of both depends upon composition of the bilayer and methods of their preparation.
- Both increase bioavailability and decrease the body clearance.

### 1.4.8. Liposome *versus* Niosome:

Niosomes are now widely studied as an alternative to liposomes, which exhibit certain disadvantages such as –they are expensive, their ingredients like phospholipids are chemically unstable because of their predisposition to oxidative degradation, they require special storage and handling and purity of natural phospholipids is variable. The differences between liposomes and niosomes are described in Table 1.4

**Table 1.4:** Comparison of Niosomes with liposomes

| NIOSOMES | LIPOSOMES |
|---|---|
| ➢ Less expensive<br>➢ But non-ionic surfactants are stable toward this<br>➢ No special methods are required for such formulations comparatively<br>➢ Non-ionic surfactants are uncharged | ➢ More expensive<br>➢ Phospholipids are prone to oxidative degradation<br>➢ Required special method for storage, handling and purification of phospholipids<br>➢ Phospholipids may be neutral or charged |

### 1.4.9. Therapeutical potentials:

- Niosomes in Oncology.

- Targeting of bioactive agents:
    a) To reticulo-endothelial system (RES)
    b) To organs other than RES
- Niosomes in leishmaniasis.
- Niosomes as carriers for Hemoglobin.
- Niosomes as diagnostic imaging.
- Transdermal delivery of drugs by niosomes.
- Niosomes as immunological adjuvant.
- Niosomes as oral drug delivery niosomes.

**1.5. Cancer:**

*(Stanley R. Hamiton, et al., 2000; Heinz Lullmam, et al., 2000; Goodman and Gillman's, 2008: http://www. Cancer.org; http:// www.en. Wikipedia.org)*

Cancer is a class of diseases characterized by out-of-control cell growth. There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected. Cancer harms the body when damaged cells divide uncontrollably to form lumps or masses of tissue called tumors.

Cancer is a complex set of diseases. Each cancer is unique in the way it grows and develops, its chances of spreading, the way it affects one`s body and the symptoms one may experience. Several factors, including location and how the cancerous cells appear under the microscope, determine how cancer is diagnosed. All cancers, however, fall into one of four broad categories.

**Carcinoma:**

Carcinoma is a malignant neoplasm of epithelial origin. It is a tumor that arises in the tissues that line the body`s organs like the nose, the colon, the penis, breasts, prostrate, urinary bladder, and the ureter. About 80% of all cancer cases are carcinomas.

**Sarcoma:**

Sarcomas are tumors that originate in bone, muscle, cartilage, fibrous tissue or fat. Ewing sarcoma (Family of tumors) and Kaposi`s sarcoma are the common types of sarcomas. Ewing sarcoma occurs during the rapid bone growth that generally occurs during puberty. The tumor may arise in the long bones of the extremities, most often in the femur (thigh bone) or the pelvis. It may also develop in the skull or the flat bones of the trunk. This type of tumor is almost never seen in black children.

**Leukemias:**

Leukemias are cancers of the blood or blood-forming organs. When leukemia develops, the body produces a large number of abnormal blood cells. In most types of leukemia, the abnormal cells are white blood cells.

The leukemia cells usually look different from normal blood cells, and they do not function properly. Leukemia can either be acute or chronic. Leukemia cells are abnormal cells that cannot do what normal blood cells do. They cannot help the body fight infections.

**Lymphomas:**

Lymphomas affect the lymphatic system, a network of vessels and nodes that

acts as the body`s filter. The lymphatic system distributes nutrients to blood and tissue, and prevents bacteria and other foreign "invade.

### 1.5.1. Burden caused by Cancer:

Cancer is a group of diseases with similar characteristics, which can occur in all living cells in the body and different cancer types have different natural history. The myth that cancer affects people mostly in the developed countries is being broken by the fact that, of the 10 million new cancer cases seen each year worldwide, nearly 5.5 million are in the less developed countries. Cancer is the second most common cause of death in the developed world and a similar trend has emerged in the developing countries too.

Cancer prevalence in India is estimated to be around 2.5 million, with over 8,00,000 new cases and 5,50,000 deaths occurring each year due to this disease. More than 70% of the cases report for diagnostic and treatment services in the advanced stages of the disease, which has lead to a poor survival and high mortality rate.

The impact of cancer is far greater than mere numbers. Its diagnosis causes immense emotional trauma and its treatment, a major economical burden, especially in a developing country like India.

The initial diagnosis of cancer is perceived by many patients as a grave event, with more than one-third of them suffering from anxiety and depression. Cancer is equally distressing for the family as well. It could greatly affect both the family’s daily functioning and economic situation. The economic shock often includes both the

loss of income and the increase of expenses because of the treatment and health care.

**Various types of Cancers:**

- **Acute lymphoblastic leukemia** (**ALL**) is a form of leukemia, or cancer of the white blood cells characterized by excess lymphoblasts. Malignant, immature white blood cells continuously multiply and are overproduced in the bone marrow. ALL causes damage and death by crowding out normal cells in the bone marrow, and by spreading (infiltrating) to other organs.

- **Acute myeloid leukemia** (**AML**), also known as **acute myelogenous leukemia**, is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age.

- **A brain tumor**, or tumour, is an intracranial solid neoplasm, a tumor (defined as an abnormal growth of cells) within the brain or the central spinal canal. Brain tumors include all tumors inside the cranium or in the central spinal canal. They are created by an abnormal and uncontrolled cell division, usually in the brain itself, but also in lymphatic tissue, in blood vessels, in the cranial nerves, in the brain envelopes (meninges), skull, pituitary gland, or pineal gland. Within the brain itself, the involved cells may be neurons or glial cells (which include astrocytes, oligodendrocytes, and ependymal cells). Brain tumors may also spread from cancers primarily located in other organs (metastatic tumors).

- **Hodgkin's lymphoma**, previously known as **Hodgkin's disease**, is a type of lymphoma, which is a cancer originating from white blood cells called lymphocytes.

It was named after Thomas Hodgkin, who first described abnormalities in the lymph system in 1832. Hodgkin's lymphoma is characterized by the orderly spread of disease from one lymph node group to another and by the development of systemic symptoms with advanced disease.

- **Kaposi's sarcoma** (**KS**) is a tumor caused by Human herpesvirus 8 (HHV8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV). It was originally described by Moritz Kaposi (KA-po-she), a Hungarian dermatologist practicing at the University of Vienna in 1872.

- **Hepatocellular carcinoma** (HCC, also called **malignant hepatoma**) is the most common type of liver cancer. Most cases of HCC are secondary to either a viral hepatitis infection (hepatitis B or C) or cirrhosis (alcoholism being the most common cause of hepatic cirrhosis).

- **Lung cancer** is a disease characterized by uncontrolled cell growth in tissues of the lung. If left untreated, this growth can spread beyond the lung in a process called metastasis into nearby tissue and, eventually, into other parts of the body. Most cancers that start in lung, known as primary lung cancers, are carcinomas that derive from epithelial cells. The main types of lung cancer are small-cell lung cancer (SCLC), also called oat cell cancer, and non-small-cell lung cancer (NSCLC).

- **Thymoma** is a tumor originating from the epithelial cells of the thymus. Thymoma is an uncommon tumor, best known for its association with the neuromuscular disorder myasthenia gravis.

- **Uterine sarcomas**: sarcomas of the myometrium, or muscular layer of the uterus, are most commonly leiomyosarcomas.

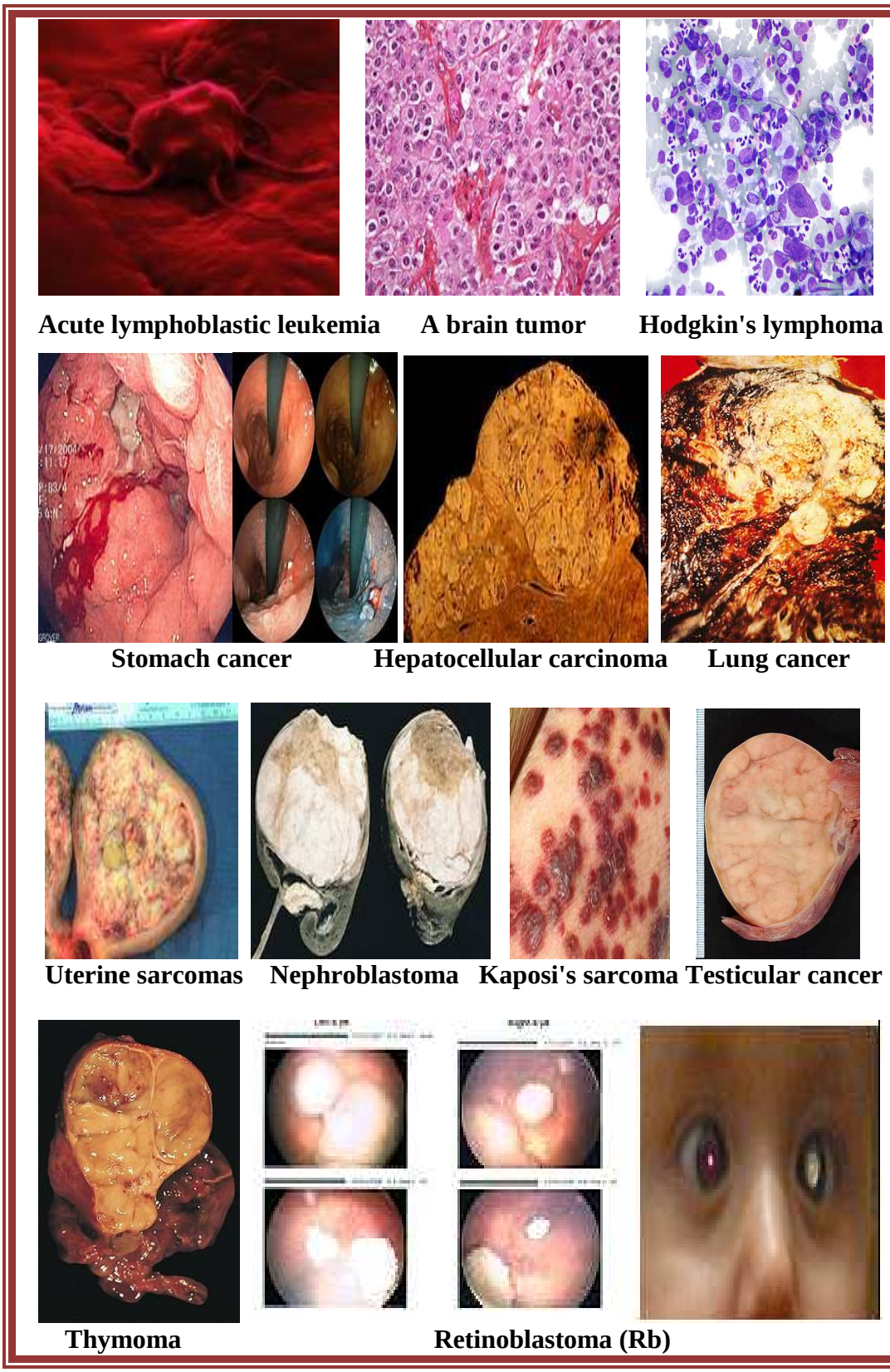



**Fig. 1.12:** Various types of Cancers

- **Retinoblastoma (Rb)** is a rapidly developing cancer that develops in the cells of retina, the light-detecting tissue of the eye. In the developed world, Rb has one of the best cure rates of all childhood cancers (95-98%), with more than nine out of every ten sufferers surviving into adulthood.

- **Kidney cancer** is a type of cancer that starts in the cells in the kidney. The two most common types of kidney cancer are renal cell carcinoma (RCC) and urothelial cell carcinoma (UCC) of the renal pelvis. These names reflect the type of cell from which the cancer developed.

- **Testicular cancer** is cancer that develops in the testicles, a part of the male reproductive system.

- **Stomach cancer**, or **gastric cancer**, refers to cancer arising from any part of the stomach. Stomach cancer causes about 800,000 deaths worldwide per year. Stomach cancer is often asymptomatic or causes only nonspecific symptoms in its early stages.

- **Wilms' tumor** or **nephroblastoma** is cancer of the kidneys that typically occurs in children, rarely in adults. Its common name is an eponym, referring to Dr. Max Wilms, the German surgeon (1867–1918) who first described this kind of tumor.

- **Neuroblastoma (NB)** is the most common extracranial solid cancer in childhood and the most common cancer in infancy. It most frequently originates in one of the adrenal glands, but can also develop in nerve tissues in the neck, chest, abdomen, or pelvis. It is a neuroendocrine tumor, arising from any neural crest element of the sympathetic nervous system or SNS.

### 1.5.2. Risk Factors:

According to epidemiological studies, 80-90% of all cancers are due to environmental factors of which, lifestyle related factors are the most important and preventable. The major risk factors for cancer are tobacco, alcohol consumption, infections, dietary habits and behavioral factors. Tobacco consumption, either by way of chewing or smoking accounts for 50% of all cancers in men. Dietary practices, reproductive and sexual practices account for 20-30% of cancers. Studies have shown that appropriate changes in lifestyle will reduce the mortality and morbidity caused to cancer. This offers the prospect for initiating primary and secondary prevention measures for control and prevention of cancers.

### 1.5.3. Types of treatment:

The most common cancer treatments include surgery, radiation therapy, and chemotherapy. These therapies may be used either alone or in combination with other therapies. Palliative treatment is treatment given to relieve symptoms of cancer and cancer treatment, such as pain. Other cancer treatment options include targeted therapy, immunotherapy, hormonal therapy, and stem cell/bone marrow transplantation. More information on each of these treatments is given below.

The first treatment a person is given is called first-line therapy. If that treatment stops working, then a person receives second-line therapy (and in some situations, third-line therapy may be available). Adjuvant therapy is treatment that is given after the first treatment (such as chemotherapy after surgery). Neoadjuvant therapy is treatment that is given before the primary treatment (such as radiation therapy before surgery).

- **Surgery:**

Surgery is the removal of the tumor and surrounding tissue during an operation. It is the primary treatment for many types of cancer, and some cancers can be completely removed with surgery alone. The side effects of surgery depend on the type of surgery and the overall health of the person before surgery. A common side effect is pain, there are many ways to provide relief when pain and other side effects occur in most people. Surgery may also be used to confirm a diagnosis (such as with a surgical biopsy), find out the extent of the cancer (called staging), and relieve side effects (such as removing an obstruction to ease pain).

- **Radiation treatment:**

Radiation therapy is the use of high-energy x-rays or other particles to kill cancer cells. The most common type of radiation treatment is called external-beam radiation therapy, which is radiation given from a machine outside the body. This has been considered as a local treatment, as it only affects one part of the body. The goals of radiation therapy include shrinking the tumor before surgery, keeping the tumor from returning after surgery, eliminating cancer cells in other parts of the body.

Side effects from radiation therapy may include fatigue, mild skin reactions, upset stomach, and loose bowel movements. Internal radiation therapy may cause bleeding, infection, or irritation after the implant is removed. Radiation treatment does not make a person radioactive. Most side effects go away soon after treatment is finished.

- **Chemotherapy:**

Chemotherapy is the use of drugs to kill cancer cells, usually by stopping the

cancer cells' ability to grow and divide. Systemic chemotherapy is delivered through the bloodstream to reach cancer cells throughout the body. A chemotherapy regimen (schedule) usually consists of a specific number of cycles given over a set period of time.

- **Targeted therapy:**

Targeted therapy is a treatment that targets the cancer's specific genes, proteins, or the tissue environment that contributes to cancer growth and survival. This type of treatment blocks the growth and spread of cancer cells while limiting damage to normal cells, usually leading to fewer side effects than other cancer medications.

- **Immunotherapy:**

Immunotherapy (also called biologic therapy) is designed to boost the body's natural defenses to fight the cancer. It uses materials made either by the body or in a laboratory to bolster, target, or restore immune system function. The side effects of immunotherapy generally include flu-like symptoms, such as chills, nausea, and fever. Immunotherapy also includes cancer vaccines-an investigational approach to helping the immune system fight cancer.

- **Hormonal therapy:**

Several types of cancer, including some breast and prostate cancers, only grow and spread in the presence of natural chemicals in the body called hormones. Hormonal therapy treats cancer by lowering the amounts of hormones in the body. It is usually used to treat cancers of the prostate, breast, thyroid, and reproductive system.

- **Stem cell/bone marrow transplantation:**

A stem cell transplant is a medical procedure in which diseased bone marrow is replaced by highly specialized cells, called hematopoietic stem cells.

Hematopoietic stem cells are found both in the bloodstream and in the bone marrow. Today, this procedure is more commonly called a stem cell transplant, rather than bone marrow transplant, because it is the blood stem cells that are typically being transplanted, not the actual bone marrow tissue.

# LITERATURE SURVEY

# 2. LITERATURE SURVEY

## 2.1. Literature Review

**1) Farhan J. Ahmad, *et al.* (2011)** had scrutinized the effect of permeation of drug into skin by taking three different vesicle size niosomal formulations. The combined influence of 3 independent variables in the preparation of venlafaxine niosomes by the Ether Injection Method. Seventeen batches were evaluated for vesicle size and polydispersity index. Contour plots were constructed to show the effects of X1, X2 and X3 on the vesicle size and polydispersity index. Niosomes dispersion were found to be stable and preparation of niosomes using factorial design was found to be well suited and sound approach to be stable niosomal formulations.

**2) Anupriya K., *et al.* (2011)** reported acyclovir niosomes as a possible approach to deliver the drug to ophthalmic cavity. Niosomes were formed using sorbitan esters (Span 20, 40, 60, and 80) and cholesterol in different molar ratio using Reverse phase evaporation method. The formulated niosomes were characterized for their in-vitro drug release efficiency, the results indicated that more sustained release pattern can be obtained by incorporating the drug in niosomes formed with Span60

**3) Jigar Vyas, *et al*. (2011)** entrapped Benzoyl peroxide into niosomes by thin film hydration technique to enhance skin penetration as well as to improve skin retention of drug, and optimized the various process parameters by partial factorial design. The optimized niosomal formulation was incorporated into HPMC K15 gel and extensively characterized for percentage drug entrapment (PDE) and in vitro

release performance. The stability of the formulation was studied at different temperatures. The study demonstrated prolongation of drug release, increased drug retention into skin, and improved permeation across the skin after encapsulation of benzoyl peroxide into niosomal topical gel.

4) **Chawda H. Singh, *et al.* (2011)** had designed noisome of Nimesulide and evaluated the vesicle size, encapsulation efficiency, in vitro release and physical stability of the system. The niosomes prepared by lipid film hydration method were multilamellar vesicles and niosomes prepared by ether injection technique were unilamellar vesicles. The physical stability study revealed that niosomal preparation stored at refrigerated temperature for 60 days had retained maximum drug for all the formulation compared to room temperature and elevated temperature conditions. The investigations conclusively demonstrated prolongation of drug release at a constant and controlled rate after niosomal encapsulation of nimesulide.

5) **Vyas Jigar, *et al*. (2011)** had formulated erythromycin niosomes by thin film hydration technique and various process parameters were optimized by partial factorial design. The optimized niosomal formulation was incorporated into carbopol gel and extensively characterized for Percentage Drug Entrapment (PDE) and in-vitro release performance. The formulations were subjected to stability study at different temperatures.

6) **Lakshmi P K., *et al.* (2011)** had prepared topical niosomal Urea gel using chitosan polymer by both lipid layer hydration and transmembrane pH gradient method. Niosomes were characterised for various physical characters. The irritancy test was performed by human repeated insult patch test (HRIPT). PASI scoring was used to determine the severity of the lesion. Niosomes prepared using span 60 showed

a better entrapment than other spans. Both the niosomes showed uniform particle size distribution. SEM analysis showed smooth outer surface. A short-term stability studies showed that niosomal gel had better stability followed by niosomes prepared using transmembrane method and lipid layer hydration method.

**7) Vedha Hari B.N., *et al.* (2011)** acquired Orlistat niosomes from proniosome to improve its poor and variable oral bioavailability. The non-ionic surfactant vesicles are prepared by the reverse phase evaporation technique (slurry method). FT-IR data revealed that, compatible and there were no interactions between the drug and excipients added in the formulation. SEM images of niosomes with various magnifications revealed the mean size of the niosomes were 100 nm with smooth surface. Niosome formulation had showed appropriate stability for 90 days by storing the formulation at room temperature. They concluded that niosomal formulations could be a promising delivery system for Orlistat with improved oral bioavailability, stability and for sustained drug release.

**8) Sambhakar S., *et al.* (2011)** had prepared niosomes containing Cefuroxime axetil by film formation method. It is characterised by SEM for particle size and morphology. The vesicle size and polydispersity index was very low. The in-vitro release study indicated the controlled release profile of niosomes. In-vitro absorption study by everted-sac method showed that maximum absorption was found. Stability study indicated that on incorporation of bile salt upto 7.5 mM in the vesicle as integral component, the stability was maximum on exposure to 20 mM bile salt, whereas they omitted incorporation of bile salt in vesicles, it showed least stability.

**9) Magharla D., *et al.* (2010)** had fabricated poly (ε-caprolactone) microspheres of etoposide by oil in water solvent evaporation technique. The results of optimized formulations showed a narrow size distribution and smooth surface. They observed microspheres prepared with of 1:10 d/p ratio and 0.5% of PVA have a high encapsulation of drug, 65.1% and the *in vitro* release profile showed a sustained etoposide release up to 87.66% at the end of 6 weeks. The DSC and the FTIR analysis showed the absence of any potent incompatibility between the drug and the polymer.

**10) Kandasamy Ruckmani and veintramuthu sankar. (2010)** had formulated zidovudine niosomes and optimized by altering the proportion of tween, span and cholesterol. The developed niosomes were evaluated for the effect of process related variables like hydration time, sonication time, charge inducing agent, centrifugation and rotational speed of evoparation flask on zidovudine entrapment and release from niosomes. Zidovudine niosome formulated with tween 80 entrapped high amount of drug and the addition of DCP enhanced drug release for a longer time (88.72% over 12h).

**11) Meenakshi B., *et al.* (2010)** had prepared different batches of metoprolol tartrate niosomes by changing the surfactant concentration but keeping the cholesterol concentration constant. The prepared niosomes were characterized for particle size, entrapment efficiency and drug release studies. They observed that Span 60 based formulations have higher entrapment efficiency than other formulations. Also Span 80 based formulations produced vesicles of smallest size and maximum cumulative percent drug release.

**12) Pavalarani N., *et al.* (2010)** had formulated niosomes of rifampicin and gatifloxacin were prepared by lipid hydration technique using rotary flash evaporator. The formulated rifampicin and gatifloxacin niosomes showed a vesicle size in the range of 100-300nm, the entrapment efficiency were 73% and 70% respectively. The *invitro* release study showed that 98.98% and 97.74% of release of rifampicin and gatifloxacin niosomes.

**13) Yan-Zhuo Zhang, *et al.* (2010)** had prepared stealthy etoposide proliposomes and studied the pharmacokinetics in rabbits. Blank stealthy liposome was prepared by film dispersion method and stealthy etoposide liposomes were prepared by using the ammonium sulfate gradient loading procedure. Encapsulation efficiency of stealthy etoposide proliposomes was determined by Sephadex chromatography. The pharmacokinetics in rabbits was evaluated by comparison with etoposide injection and conventional liposomes. The liposomes were round or oval. Intravenous injection was administered at a dose of 1.5 mg/kg Etoposide, the three kinds of etoposide preparations were fitted with the two-compartment model. Results indicated that the stealthy etoposide proliposomes could significantly extend the duration of Etoposide in blood circulation.

**14) Abdul Hasan Sathali A., *et al.* (2010)** had developed a targeted delivery to the fungal affected cells. Niosomes of terbinafine hydrochloride were formulated by thin film hydration method using different ratios of non ionic surfactant (tween 20, 40, 60, and 80) and cholesterol with constant drug concentration. The prepared formulations were evaluated for its vesicle size (by AFM), entrapment efficiency (by dialysis method) *in vitro* release studies and antifungal activities. Increase in surfactant concentration, increased the entrapment efficiency (up to 84.92%) and the

formulation with surfactant cholesterol ratio 2:1 in each group of surfactant showed good entrapment.

**15) Pandey Shivanand, *et al*. (2010)** had developed Cefpodoxime proxetil niosomes by using incorporation of synthetic non-ionic surfactant with cholesterol. The encapsulated niosomes were characterized in terms of vesicle shape using a scanning electron microscope, particle size determination by optical microscopy, compatability studies by Fourier transform infrared spectroscopy, in-vitro drug release studies, kinetics of drug release and stability studies of prepared niosomes.

**16) Gyanendra singh, *et al.* (2010)** had prepared rifampicin niosome by reverse phase evopration method and changed by charge imducing agent (dicetyl phosphate).niosome were characterized by determining particle size, polydispersity index (PI), zeta potential and scanning electron microscopy. The result of study indicates that the investigated system has potential to remain in the desired site for prolonged period and is capable of maintaining a constant drug concentration for a longer duration.

**17) Lakshmi P K., *et al.* (2009)** had formulated niosomes of salbutamol sulphate using Span 60 as the surfactant, by employing different techniques namely, thin film hydration, hand shaking, ether injection, lipid layer hydration and transmembrane pH gradient method. The drug encapsulation efficiency varied from 62 % to 87 %. Transmembrane pH gradient method was found to be most satisfactory which released 78.4 % of drug in 24 h. The lyophilized formulation was characterized by infrared spectroscopy and conducted tissue distribution studies in albino rats and bio- availability studies in rabbits.

**18) Pratap S J., *et al.* (2009)** had developed nonionic surfactant vesicles (niosomes) to improve poor and variable oral bioavailability of Griseofulvin by thin film method and ether injection method using different nonionic surfactants span 20, span 40, and span 60. Result indicated that the niosomes prepared by thin film method with span 60 provided higher entrapment efficiency. The niosomal formulation exhibited significantly retarded in- vitro release and in- vivo study revealed that the niosomal dispersion significantly improved the oral bioavailability of griseofulvin in albino rats after a single oral dose. Plasma drug profile also suggested that the developed niosomal system also has the potential of maintaining therapeutic level of griseofulvin for a longer period of time as compared to free griseofulvin.

**19) Ghada Abdelbary, *et al.* (2008)** had investigated the feasibility of using non-ionic surfactant vesicles (niosomes) as carriers for the ophthalmic controlled delivery of a water soluble local antibiotic; Gentamicin sulphate. The ability of these vesicles to entrap the studied drug was evaluated by determining the entrapment efficiency %EE after centrifugation and separation of the formed vesicles. In-vitro drug release results confirmed that niosomal formulations have exhibited a high retention of gentamicin sulphate inside the vesicles such that there in vitro release was slower compared to the drug solution. Ocular irritancy test performed on albino rabbits, showed no sign of irritation for all tested niosomal formulations.

**20) Doijad R.C., *et al.* (2008)** had formulated niosome of cisplatin by ether injection method using spans and tweens. They observed highest cumulative percent drug release with span-60 (96.87%) and with tween -20 (70.49%) in 12 hours. The in

–vivo result that the drug is preferentially targeting to liver followed by spleen and lungs.

**21) Varaporn Buraphacheep Junyaprasert, *et al.* (2008)** had examined an influence of different types of membrane additives including negative charge (dicetylphosphate, DCP), positive charge (stearylamine, STR) and non-ionic molecule (cholesteryl poly-24-oxyethylene ether, SC24) on the physicochemical properties of drug-free and drug-loaded niosomes. The results showed that incorporation of salicylic acid to the niosomes did not affect zeta potential values, but addition of the membrane additives changed the zeta potential depending on the type of the additives. Transmission electron microscopy revealed that acid after 3 months of storage indicating the good stability.

**22) Manivannan Rangasamy, *et al.* (2008)** had formulated acyclovir entrapped niosomes by hand shaking and ether injection process with different ratios of (1:1, 1:2 and 1:3) cholesterol (CHOL) and Span-80 (Non-ionic surfactant).The niosomes prepared were in the size range of 0.5- 5 microns in the case of hand shaking process and 0.5-2.5 microns in the case of Ether injection process. The order of encapsulation efficiency increases when span-80 concentration was increased. In-vitro release study on acyclovir niosomes indicates 76.64% release for formulation prepared with CHOL: Span-80 (1:1) and it takes an extended period of 1 day and 16 h for release.

**23) Roopa Karki, *et al.* (2008)** had encapsulated Isoniazid as niosomal formulation using ethanol injection method. A different ratio of cholesterol was used. The formulated systems were characterized for in-vitro by size distribution analysis,

drug entrapment efficiency, drug release profiles and in-vivo drug disposition was evaluated in normal, healthy albino rats. In vitro release pattern indicated that about total drug content were released within 48 h. The drug disposition by niosomal drug delivery proved that the drug accumulated in visceral organs like lung, kidney, liver and spleen. This proved that niosomal drug delivery system has lesser toxicity than free drug.

**24) Sanaa A. EL-Gizawy, *et al.* (2007)** had designed acyclovir niosomes to improve its poor and variable oral bioavailability by the conventional thin film hydration method. The percentage entrapment was ~11% and most of the niosomes had unilamellar spherical shape. The niosomal formulation exhibited significantly retarded release compared with free drug. The in-vivo study revealed that the niosomal dispersion significantly improved the oral bioavailability of acyclovir in rabbits after a single oral dose of 40 mg $kg^{-1}$.

**25) Balasubramanian A., *et al.* (2002)** had developed niosome carrying anti neoplastic agent Daunorubucin hydrochloride, used in the treatment of acute myelocytic leukemia and acute lymphocytic leukemia. The release of the drug from the vesicle in – vitro was highly prolonged when compared to the free drug and around 22 ½ has more when compared to the free drug.

**26) David G. Rhodes and Almira I. (2001)** urbanized proniosomes with a maltodextrin carrier, which provides for rapid reconstitution of niosomes with minimal residual carrier. SEM images of proniosomes with various degrees of surfactant loading and images of pure surfactant were compared. Direct observation and particle size measurements by laser light scattering provided characterization of the final niosome preparations. The appearance of a coarse, broken surface on the

proniosomes correlates with inefficient rehydration and occurrence of aggregation and precipitate in the final niosome suspension. These observations provided an indication of the requirements for dry proniosomes to yield niosome suspensions of high quality.

**27) Gayathri Devi S., *et al.* (2000)** had formulated niosome of sumatripan succanite using lipid hydration method. The prepared noisome were evaluated for entrapment efficiency, size analysis in vitro release studies and nasal absorption using an ex vivo animal succinate over a period of 6 hour and 98.1% of the drug absorbed from the nasal mucosa ex vivo.

**28) Popli H and Nari M.S. (1996)** had described the encapsulation of tenoxicam in niosome and invetigated the influence of the varying proportion of surfactant, cholesterol and dicetyl phosphate on the morphology, particle size distribution, entrapment efficiency and in – vitro drug release of noisome.

**29) Azmin M. N., *et al.* (1986)** had formulated methotrexate niosomes and studied the effect of niosome encapsulation on the metabolism and urinary and faecal excretion of methotrexate (MTX) in mice following oral and intravenous administration, and compared with the effects of co-administration of free drug and polysorbate 80, which does not form vesicles. The entrapped noisome observed to reduce the excretion of MTX into urine and bile whereas free polysorbate 80 increased its excretion. They monitored of the levels of MTX and its 7-hydroxy metabolite which indicated that entrapped MTX is protected from rapid metabolism**.**

## 2.2. DRUG PROFILE

*(IP, 2007; USP, 2007; Goodman and Gillman's, 2008; Sweetman S.C., Martindale, 2009; http:// www.google.com, drug bank; http://www.cancer.org)*

**ETOPOSIDE:**

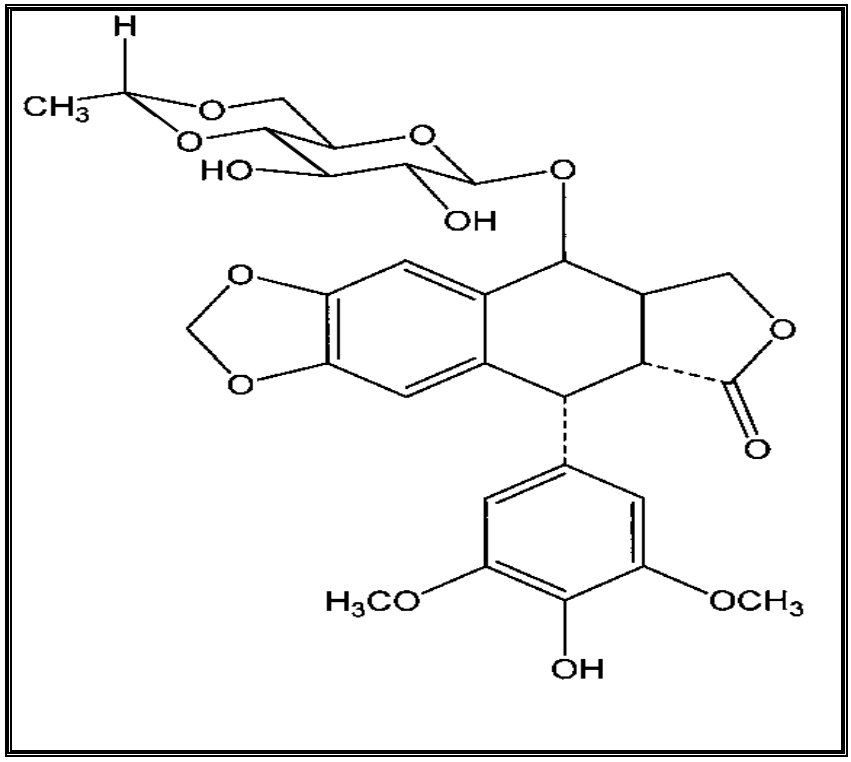
Etoposide is a semi-synthetic derivative of podophyllotoxin.

1. **Synonyms** **:** (-)-Etoposide, Etoposidum [INN-Latin], trans-Etoposide.
2. **Molecular formula** **:** $C_{29}H_{32}O_{13}$
3. **Molecular weight** **:** 588.7
4. **IUPAC Name** **:** (10R,11R,15R,16S)-16- {[(2R,4aR,6R,7R,8R,8aS)-7,8dihydroxy2methyl-hexahydro-2H-pyrano[3,2-d][1,3] dioxin-6-yl]oxy}-10-(4-hydroxy-3,5-dimethoxyphenyl)-4,6,13-trioxatetracyclo[7.7.0.0^{3,7}.0^{11,15}] hexadeca-1(9),2,7-trien-12-one.
5. **Chemical name** **:** 4′-demethylepipodophyllotoxin-9-[4,6-*O*ethylidene- β-D-glucopyranoside]
6. **CAS number** **:** 33419-42-0

**7. Category** **:** Anti-cancer drug.

**8. Melting point** **:** 236-251°C

**9. Structural formula:**




**10. Solubility:**

- Slightly soluble in water, slightly soluble in alcohol and chloroform,
- In ethyl acetate, in methylene chloride, sparingly soluble in methanol**.**

**11. Description:**

- White to yellow-brown crystalline powder;
- White to off-white crystalline.

**12. Pharmacokinetic data:**

- **Bioavailability** : Highly variable, 25 to 75%
- **Protein binding** : 97%
- **Metabolism** : Hepatic (CYP3A4 involved)
- **Half-life** : Oral: 6 h., IV: 6-12 h., IV in children: 3 h.
- **Excretion** : Renal and fecal
- **Pka** : 9.8
- **logP** : 1.16
- **Nature** : Hydrophobic

**13. Dosage and Administration:**

**Table 2.1:** Table indicating dosing Forms & Strengths

| S.NO | DOSAGE FORMS | STRENGTHS |
|---|---|---|
| 1 | Capsules | 50mg |
| 2 | Injectable solution | 20mg/mL |
| 3 | Powder for injection | 100mg |

- **Adult Dosing & Uses:**
  - **Testicular Cancer:**
    - 50-100 mg/sq.meter/d IV on days 1-5, OR
    - 100 mg/sq.meter/d IV on days 1, 3, 5.Repeat 3-4week.

- **Small-Cell Lung Cancer (SCLC):**
  - 35 mg/sq.meter/d IV x 4 d, OR
  - 50 mg/sq.meter/d IV x 5 d; repeat q3-4week.
- **Epithelial ovarian cancer**:
  - 50 mg/m2 daily on days 1 through 21.
- **Germ cell ovarian cancer**:
  - 100 mg/m2 IV on days 1 through 5.
- **Hodgkin's Disease:**
  - 100mg/m2/day on days 1, 2 and 3.
  - Total Dose/Cycle = 300 mg/m2.

**14. Mechanism of action:**

Etoposide inhibits DNA topoisomerase II, thereby inhibiting DNA re-ligation. This causes critical errors in DNA synthesis at the premitotic stage of cell division and can lead to apoptosis of the cancer cell. Etoposide is cell cycle dependent and phase specific, affecting mainly the S and G2 phases of cell division**.**

**15. Pathway:**

Etoposide is a podophyllotoxin derivative that is used in the treatment of certain cancers. It inhibits mitosis and induces cell death by acting as a topoisomerase

II poison. Topoisomerase II is an enzyme in the nucleus of cells that unwinds DNA by making transient double-stranded breaks, relieving the torsion of supercoiled DNA. In the unwound form, DNA can serve as a template for DNA replication as well as transcription. In the normal state, this effect is transient and the breaks DNA are quickly religated by topoisomerase II itself. Etoposide, however, inhibits religation and stabilizes the DNA-topoisomerase II complex in the cleaved DNA form, ultimately leading to breaks in both DNA chains and cell death. Etoposide is also converted into catechol and o-quinone derivatives in the liver and in lysosomes respectively. These metabolites are highly oxidative and can directly damage DNA, which may also contribute to the drug's cytotoxic effects.

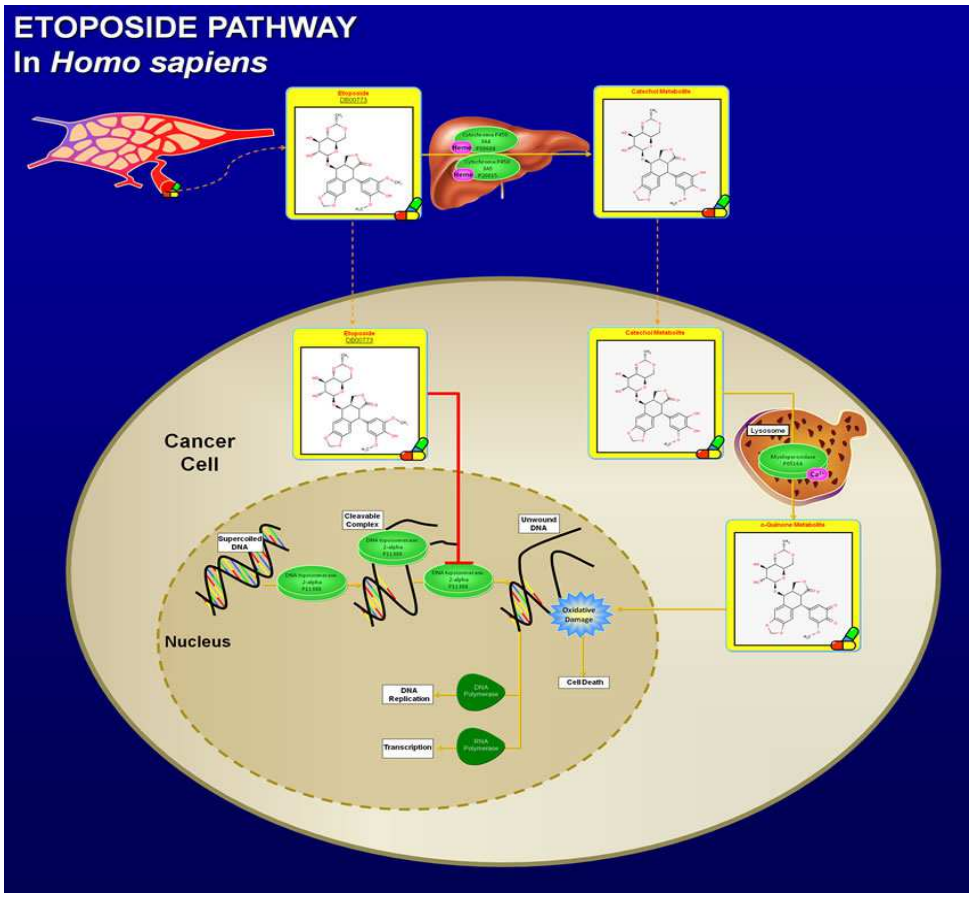



**Fig. 2.1:** Schematic representation of Etoposide pathway

**16. Therapeutic uses:**

- Autoimmune deficiency syndrome (AIDS)—associated Kaposi's sarcoma (a type of cancer of the skin and mucous membranes that is more common in patients with AIDS).

- Cancer in the bone.
- Cancer of the adrenal cortex (the outside layer of the adrenal gland).
- Cancer of the endometrium.
- Cancer of the lung (a certain type of lung cancer usually associated with prior smoking, passive smoking, or radon exposure).
- Cancer of the lymph system (a part of the body's immune system) that affects the skin.
- Cancer of the ovaries (a type of cancer found in the egg-making cells).
- Cancer of the stomach.
- Cancer of unknown primary site.
- Cancers of the blood and lymph system.
- Cancers of the soft tissues of the body, including the muscles, connective tissues (tendons), vessels that carry blood or lymph, or fat.
- Ewing's sarcoma (a type of cancer found in the bone).
- Gestational trophoblastic tumors (tumors in the uterus or womb).
- Hepatoblastoma (a certain type of liver cancer that occurs in children).
- Multiple myeloma (a certain type of cancer of the blood).
- Myelodysplastic syndromes (MDS).
- Neuroblastoma (a cancer of the nerves that usually occurs in children).

- Retinoblastoma (a cancer of the eye that usually occurs in children).
- Thymoma (a cancer of the thymus, which is a small organ that lies under the breastbone).
- Tumors in the brain.
- Wilms' tumor (a cancer of the kidney that usually occurs in children).
- Testicular cancer.

**17. Toxicities:**

❖ **Haematological Toxicity** :

The principal toxicity of etoposide is dose-related bone marrow suppression, Leucopenia was observed in 60 to 91 percent and 7 to 17 percent, of patients**,** Thrombocytopenia.

❖ **Gastrointestinal Toxicity**:

Nausea and vomiting are the major gastrointestinal toxicities.

❖ **Other Toxicities**:

The following reactions have been rarely reported:

- Central nervous system toxicity (somnolence and fatigue)
- Liver toxicity (transient jaundice and elevated alkaline phosphatase),
- Renal toxicity (elevated urea; hyperuricaemia),
- Septicaemia during high dose regimens

**18. Side effects:**

**Table 2.2:** Side effects of Etoposide

| COMMON | LESS COMMON | RARE |
|---|---|---|
| ♦ Low white blood cell count with increased risk of infection<br>♦ Low blood platelet count with increased risk of bleeding<br>♦ Nausea and vomiting<br>♦ Loss of appetite<br>♦ Hair loss, including face and body hair | ♦ Constipation<br>♦ Diarrhoea<br>♦ Fever and chills<br>♦ Lowered red blood cell count (anemia) | ♦ Low blood pressure while drug is being given<br>♦ Sores in mouth and throat<br>♦ Changes in how foods taste<br>♦ Rash, which can become serious<br>♦ Itching<br>♦ Numbness and tingling in hands and/or feet<br>♦ Allergic reactions (may include chills, fever, rapid heart rate, trouble breathing, dizziness)<br>♦ Increased risk of a second cáncer. |

**19. Drug interactions:**

Cyclosporine may increase blood levels of etoposide if taken at the same time. Any drug that can increase your risk of bleeding may pose a hazard while your blood counts are low. Aspirin, ibuprofen, naproxen, and other pain relievers and fever reducers can have this effect. Blood thinners such as warfarin may also interact.

**20. Precautions:**

In all instances where the use of Etoposide is considered for chemotherapy, the physician must evaluate the need and usefulness of the drug against the risk of adverse reactions. Most such adverse reactions are reversible if detected early. If severe reactions occur, the drug should be reduced in dosage or discontinued and appropriate corrective measures should be taken. Reinstitution of Etoposide therapy should be carried out with caution and with adequate consideration of the further need for the drug and alertness as to possible recurrence of toxicity.

**Laboratory Tests:** Periodic complete blood counts should be done during the course of Etoposide treatment. They should be performed prior to each cycle of therapy and at appropriate intervals during andafter therapy. At least one determination should be done prior to each dose of Etoposide.

**Renal Impairment:** In patients with impaired renal function, the initial dose modification should be considered based on measured creatinine clearance.

**21. Brand names :**

- Eposin
- Etopophos
- Lastet
- Toposar
- Vepesid
- Vepesid J
- Zuyeyidal

**22. Storage:** Store protected from moisture.

## 2.3.EXCIPIENTS PROFILE

### 2.3.1. CHOLESTEROL: *(USP, 2009; Raymond C. R., 2006)*

**1. Nonproprietary Names:**

- ❖ BP: Cholesterol
- ❖ JP: Cholesterol
- ❖ PhEur: Cholesterolum
- ❖ USPNF: Cholesterol

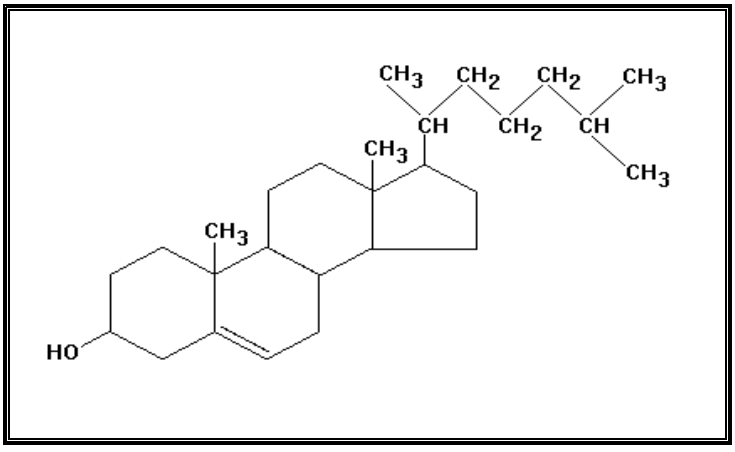
**2. Synonyms** **:** Cholesterin; cholesterolum.

**3. Chemical Name and CAS Registry Number :** Cholest-5-en-3b-ol [57-88-5]

**4. Empirical Formula** **:** $C_{27}H_{46}O$

**5. Molecular Weight** **:** 386.67

**6. Structural Formula :**



**7. Functional Category:**

Emollient; emulsifying agent.

**8. Applications in Pharmaceutical Formulation or Technology:**

Cholesterol is used in cosmetics and topical pharmaceutical formulations at concentrations of 0.3–5.0% w/w as an emulsifying agent. It imparts water-absorbing power to an ointment and has emollient activity. Cholesterol also has a physiological role. It is the major sterol of the higher animals, and it is found in all body tissues, especially in the brain and spinal cord. It is also the main constituent of gallstones.

**9. Description:**

Cholesterol occurs as white or faintly yellow, almost odorless, pearly leaflets, needles, powder, or granules. On prolonged exposure to light and air, cholesterol acquires a yellow to tan color.

**10. Typical properties:**

- **Boiling point:** 360ºC
- **Density:** 1.052 g/cm$^3$ for anhydrous form.
- **Melting point:** 147–150ºC
- **Loss on Drying:** Not more than 0.3%
- **Residue on ignition:** Not more than 0.1%
- **Specific rotation:** Between 34º and 38º

❖ **Solubility of cholesterol:**

**Table 2.3:** Solubility of cholesterol in various

| S.NO | SOLVENT | SOLUBILITY AT 20ºC<br><br>UNLESS AND OTHERWISE STATED |
|---|---|---|
| 1. | Acetone | Soluble |
| 2. | Benzene | 1 in 7 |
| 3. | Chloroform | 1 in 4.5 |
| 4. | Ethanol | 1 in 147 at 0ºC<br>1 in 78 at 20ºC<br>1 in 29 at 40ºC<br>1 in 19 at 50ºC<br>1 in 13 at 60ºC |
| 5. | Ethanol (95%) | 1 in 78 (slowly)<br><br>1 in 3.6 at 80ºC |
| 6. | Ether | 1 in 2.8 |
| 7. | Hexane | 1 in 52 |
| 8. | Isopropyl myristate | 1 in 19 |
| 9. | Methanol | 1 in 294 at 0ºC<br>1 in 153 at 20ºC<br>1 in 53 at 40ºC<br>1 in 34 at 50ºC<br>1 in 23 at 60ºC |
| 10. | Vegetable oils | Soluble |
| 11. | Water | Practically insoluble |

**11. Method of Manufacture:**

The commercial material is normally obtained from the spinal cord of cattle by extraction with petroleum ethers, but it may also be obtained from wool fat. Purification is normally accomplished by repeated bromination. Cholesterol may also be produced by entirely synthetic means. Cholesterol produced from animal organs will always contain cholestanol and other saturated sterols.

**12. Stability and Storage Conditions:**

Cholesterol is stable and should be stored in a well-closed container, protected from light.

### 2.3.2. POLYSORBATE 80: *(USP, 2009; Raymond C. R., 2006)*

**1. Definition:**

Mixture of partial esters of fatty acids, mainly *Oleic acid (0799)*, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole ofsorbitol and sorbitol anhydrides.

**2. Nonproprietary Names:**

- BP: Polysorbate 80
- JP: Polysorbate 80
- PhEur: Polysorbatum 80
- USPNF: Polysorbate 80.

**3. Synonyms:**

Atlas E; Armotan PMO 20; Capmul POE-O; Cremophor PS 80; Crillet 4; Crillet 50; Drewmulse POE-SMO; Drewpone 80K; Durfax 80; Durfax 80K; E433; Emrite 6120; Eumulgin SMO; Glycosperse O-20; Hodag PSMO-20; Liposorb O-20; Liposorb O-20K; Montanox 80; polyoxyethylene 20 oleate; Protasorb O-20; Ritabate 80; (Z)-sorbitan mono-9-octadecenoate poly(oxy1,2-ethanediyl) derivatives; Tego SMO 80; Tego SMO 80V; Tween 80.

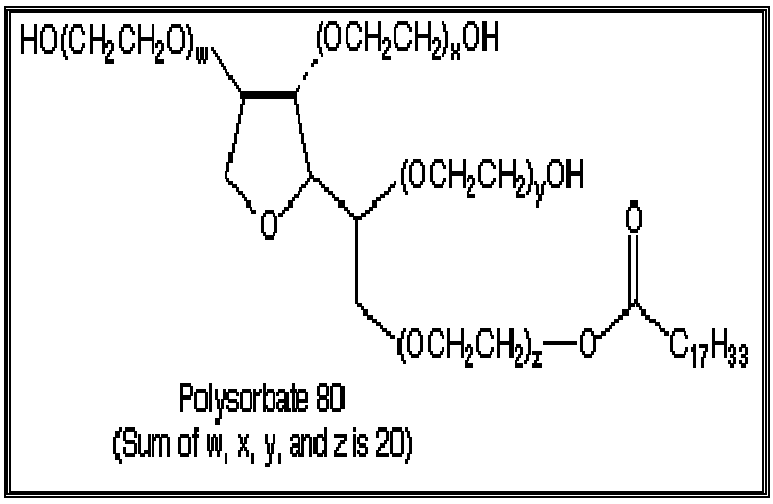
**4. Chemical Name and CAS Registry Numbers:**

Polyoxyethylene 20 sorbitan monooleate and [9005-65-6]

**5. Empirical Formula** **:** $C_{64}H_{124}O_{26}$

**6. Molecular Weight** **:** 1310

**7. Structural formula:**



**8. Functional Category:**

Emulsifying agent; nonionic surfactant; solubilizing agent; wetting, dispersing/suspending agent.

**9. Description:**

- **Appearance:** Oily, yellowish or brownish-yellow, clear or slightly opalescent, liquid.
- **Odor:** Characteristic odor and a warm.
- **Taste:** Somewhat bitter taste.

**10. Typical properties:**

- **Acidity/alkalinity** **:** pH = 6.0–8.0 for a 5% w/v aqueous solution.
- **Flash point** **:** 149ºC
- **Acid value (%)** **:** 2.0
- **Hydroxyl value** **:** 65-80
- **Moisture content** **:** 3.0
- **Saponification value** **:** 45-55
- **HLB value** **:** 15.0
- **Specific gravity at 25ºC** **:** 1.08
- **Viscosity (mPa s)** **:** 425
- **Surface tension at 20ºC (mN/m):** 42.5
- **Heavy metals** **:** 0.001%
- **Total ash** **:** Maximum 0.25 per cent

❖ **Solubility:**

- Dispersible in water, in anhydrous ethanol, in ethyl acetate and in methanol. Practically insoluble in fatty oils and in liquid paraffin.

**11. Method of Manufacture:**

Polysorbates are prepared from sorbitol in a three-step process. Water is initially removed from the sorbitol to form a sorbitan (a cyclic sorbitol anhydride). The sorbitan is then partially esterified with a fatty acid, such as oleic or stearic acid, to yield a hexitan ester. Finally, ethylene oxide is chemically added in the presence of a catalyst to yield the polysorbate.

**12. Applications in Pharmaceutical Formulation or Technology:**

Polysorbate 80 is an excipient that is used to stabilize aqueous formulations of medications for parenteral administration, and used as an emulsifier in the manufacture of the popular anti-arrhythmic amiodarone. It is also used as an excipient in some European and Canadian influenza vaccines. They have been found to be useful in improving the oral bioavailability of drug molecules that are substrates for p-glycoprotein. Polysorbates are also widely used in cosmetics and food products.

**13. Stability and Storage Conditions:**

Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

# Aim

# &

# Objective

# 3. AIM AND OBJECTIVE

The concept of drug delivery to a particular site for the treatment of localized disease in the body and thereby decreasing adverse effect of drug with improving its therapeutic index is being considered as a challenge in modern formulation design. So, constant efforts have been persued in order to design such an ideal drug delivery system which improves therapeutic index of drugs and also improves patient compliance. One such area which has attracted increasing attention of pharmaceutical scientist and has shown very promising results is “Target oriented Drug Delivery System”.

Amongst all carriers utilized for target oriented drug delivery, vesicular drug delivery system in form of liposomes and niosomes are most extensively investigated. Vesicular drug delivery systems such as liposomes and niosomes have been claimed to prolong and control the action of drug.

There are problems in the general applications of liposomes. They have problems regarding degradation due to instability of phospholipid molecules and cost of production is high. One alternative of phospholipids is the hydrated mixture of cholesterol and nonionic surfactants. This mixture provides vesicles known as niosomes or non-ionic surfactant vesicles. Thus Niosomes have been proved to be versatile carrier for wide variety of drug.

Niosome has been regarded as a beneficial carrier system for parental application of variety of drugs due to their biocompatible and biodegradable

properties. And there is an increase in their circulation time. Longer circulation of Niosome in blood resulted in higher uptake of drug in tumor.

Etoposide is a semi synthetic derivative of podophyllotoxin that exhibits anti tumor activity. A Etoposide is first line treatment in patient with small cell lung cancer & requiring the treatment over a prolonged period of time. Etoposide when loaded into niosomes could release the drug over a long period of time. Etoposide encapsulation by niosomal drug delivery system is to reduce the dosing frequency as well as patient compliance.

In present work an attempt was made to prepare the niosomes of Etoposide can continuously deliver therapeutically significance of drug for prolonged time period. To study the effect of variable on niosome performance and characteristic different batches need to be prepared by implementing $3^2$ factorial designs.

# PLAN OF WORK

# 4. PLAN OF WORK

1. **LITERATURE REVIEW**
2. **SELECTION OF DRUG AND EXCIPIENTS**
3. **PROCUREMENT OF DRUG AND EXCIPIENTS**
4. **EXPERIMENTAL WORK**

- **PREFORMULATION STUDIES**
  - **Identification of Drug**
    - Organoleptic Properties
    - Determination of Melting Point
    - Solubility Study
    - FTIR
    - UV Spectrophotometric Study
      - Determination of $\lambda_{max}$
      - Development of standard curve of Etoposide
    - Loss on drying
    - Assay of Etoposide

- **FORMULATION DESIGNING BY $3^2$ FULL FACTORIAL DESIGN**
- **FORMULATION OF NOVEL NIOSOMAL DELIVERY SYSTEM**
- **EVALUATION OF NIOSOMES**
  - Determination of niosomes recovery (%), entrapment efficiency (%EE) and drug loading efficiency (%LE)

- Shape and surface morphology
- Analysis of vesicle size and polydispersity index
- Determination of vesicle surface charge (Zeta potential)
- Compatibility study by FTIR and DSC analysis
- *In-vitro* drug release studies
- Kinetic of *in-vitro* drug release
- Sterilization technique
- Test for sterility
- In-vivo drug targeting studies
- Stability studies

5. **RESULTS AND DISCUSSION**
6. **SUMMARY AND CONCLUSION**
7. **FUTURE PROSPECTS**
8. **BIBLIOGRAPHY**

# Materials And Equipments

# 5. MATERIALS AND EQUIPMENTS

## 5.1. MATERIALS USED:

**Table 5.1:** List of Drug and Excipients with source

| S.No. | Ingredients | Supplier |
|---|---|---|
| 1 | Etoposide | Strides Arco lab, Bangalore. |
| 2 | Cholesterol | Loba Chemie Pvt. Ltd., Mumbai. |
| 3 | Polysorbate 80 | S D fine-chem limited, Mumbai. |
| 4 | Chloroform | Loba Chemie Pvt. Ltd., Mumbai. |
| 5 | Diethyl ether | Loba Chemie Pvt. Ltd., Mumbai. |
| 6 | Hydrochloric acid | S D fine-chem limited, Mumbai. |
| 7 | Disodium hydrogen phosphate | S D fine-chem limited, Mumbai. |
| 8 | Potassium dihydrogen phosphate | Qualigens fine chemicals, Mumbai. |
| 9 | Sodium hydroxide | S D fine-chem limited, Mumbai |
| 10 | Sodium chloride | Qualigens fine chemicals, Mumbai. |
| 11 | Dialysis bag | Himedia labaratories, Mumbai. |

## 5.2. EQUIPMENTS USED:

**Table 5.2:** List of Equipments with model/make

| S.No | Equipments | Model/ Make |
|---|---|---|
| 1. | Electronic balance | Shimadzu BL-220H |
| 2. | Magnetic stirrer | 1-MLH, remi equipments limited, vasai |
| 3. | Freeze drying apparatus | LYPHLOCK; Labconco, Kansas City, MO |
| 4. | Scanning electron microscope | FEI Quanta FEG 200 HR- SEM |
| 5. | Zeta potential | Zetasizer , Malvern, UK |
| 6. | Photon correlation spectroscopy | Malvern instruments, UK |
| 7. | pHmeter | LI120 pHmeter, ELICO LTD |
| 8. | Humidity chamber | Labtech |
| 9. | Micro centrifuge | RM-12C, Remi equipments limited, vasai |
| 10. | Homogenizer | Remi equipments limited, vasai |
| 11. | UV Visible spectrophotometer | Shimadzu-1700 Pharmaspec<br>UV-visible spectrophotometer, Elico |
| 12. | FTIR spectrophotometer | Shimadzu FTIR-801 spectrophotometer |
| 13. | Differential scanning calorimeter | Shimadzu DSC W70 |
| 14. | Melting point apparatus | Guna enterprises, Chennai |

# PREFORMULATION STUDIES

# 6. PREFORMULATION STUDY

## 6.1. Identification of Drug:

The preliminary studies were carried out by testing of different physical and chemical properties of drug as follows.

### 6.1.1. Organoleptic properties:

The Organoleptic properties like physical state, color, odor etc., of the drug was reported with help of the descriptive terminology. It helps to identify the drug.

### 6.1.2. Melting point: *(IP, 2007)*

It is the easy way to identify the drug. The melting point of Etoposide was tested by use of a laboratory melting point apparatus with a procedure given in the Indian Pharmacopeia 2007.

### 6.1.3. Solubility study: *(IP, 2007)*

The solubility of Etoposide was determined by micropipette method in various solvents in order to meet the official standards. The solubility of drug was recorded by using various descriptive terminology specified in Indian pharmacopoeia, 2007.

The general description of solubility as per Indian Pharmacopoeia was listed out in the Table 6.1.

**Table 6.1**: Description of solubility

| Descriptive term | Parts of solvent required for 1 part of solute |
|---|---|
| Very soluble | Less than 1 |
| Freely soluble | From 1 to 10 |
| Soluble | From 10 to 30 |
| Sparingly soluble | From 30 to 100 |
| Slightly soluble | From 100 to 1,000 |
| Very slightly soluble | From 1,000 to 10,000 |
| Practically insoluble | Greater than or equal to 10,000 |

**6.1.4. FTIR spectroscopy:** *(Skoog D.A., et al., 1996; IP, 2007)*

The infrared spectrum was generally used as an identification parameter to know the chemical structure of drugs. For the FTIR spectrum of Etoposide FTIR spectrophotometer was used.

A small quantity of sample was mixed with sufficient potassium bromide and compressed into a pellet by applying a 10 tons pressure with help of a hand operated press. This pellet was kept in a sample holder and scanned from 4000 to 400 $cm^{-1}$. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum.

### 6.1.5. UV Spectrophotometric Study:

#### 6.1.5.1. Determination of $\lambda_{max}$:

The absorption maximum of the standard solution was scanned between 200-400 nm regions on Shimadzu-1700 Pharmaspec UV-visible spectrophotometer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum.

#### 6.1.5.2. Development of standard curve of Etoposide in methanol:

- **Preparation of stock solution:**

Accurately weighed 25 mg of etoposide was dissolved in little quantity of methanol and volume was adjusted to 25 mL with the same to prepare standard solution having concentration of 1000 µg/ mL. From this 10 mL was pipette out and made up to 100 mL with methanol to produce 100 µg/mL.

- **Procedure:**

From the stock solution, aliquots of 2, 4, 6, 8 and 10 mL were transferred to 10 mL volumetric flasks and final volume was made to 10 mL with methanol to get 20 to 100 µg/mL. Absorbance values of these solutions were measured against blank (methanol) at 284 nm using UV-visible spectrophotometer.

#### 6.1.5.3. Development of standard curve of Etoposide:

- **Preparation of phosphate buffer saline pH 7.4:**

Phosphate buffer saline pH 7.4 was prepared according to I.P. 2007. A quantity of 2.38g disodium hydrogen phosphate, 0.19g potassium dihydrogen phosphate and 8.0g sodium hydroxide was diluted with fresh distilled water to produce 1000 mL.

- **Preparation of stock solution of Etoposide in PBS pH 7.4 solution:**

Accurately weighed 25 mg of etoposide was dissolved in little quantity of phosphate buffer saline solution pH 7.4 and volume was adjusted to 25 mL with the same to prepare standard solution having concentration of 1000 µg/ mL. From this 10 mL was pipette out and made up to 100 mL with PBS pH to produce 100 µg/mL.

- **Procedure:**

From the stock solution, aliquots of 2, 4, 6, 8 and 10 mL were transferred to 10 mL volumetric flasks and final volume was made to 10 mL with pH 7.4 phosphate buffer saline to get 20 to 100 µg/mL. Absorbance values of these solutions were measured against blank (phosphate buffer saline pH 7.4) at 284 nm using UV-visible spectrophotometer.

**6.1.6. Loss on drying:** *(IP, 2007)*

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified condition. The accurately weighed 1gm of sample was transferred in glass-stoppered, shallow weighing bottle and accurately weighed the bottle. The bottle was transferred in oven and substance was dried at 105°C for 4 hours. The bottle was removed from oven and reweighed; loss on drying was calculated by following equation,

$$\text{LOD} = \frac{\text{Initial weight of substance} - \text{Final weight of substance}}{\text{Initial weight of substance}} \times 100$$

**6.1.7. Assay of Etoposide:**

Accurately weighed 10 mg of etoposide was dissolved in little quantity of methanol and volume was adjusted to 100 mL with the same to prepare standard solution having concentration of 100 µg/mL. From the above solution, aliquots of 6 mL were transferred to 10 mL volumetric flasks and final volume was made to 10 mL with methanol. Absorbance values of these solutions were measured against blank (methanol) at 285 nm using Shimadzu-1700 Pharmaspec UV-visible spectrophotometer. The percentage purity of drug was calculated by using calibration graph method (least square method).

# Formulation Of Novel Niosomal Delivery System

# 7. FORMULATION OF NOVEL NIOSOMAL DELIVERY SYSTEM

## 7.1. Designing the formula:

*(Vyas Jigar, et al., 2011; Farhan J. Ahmad, et al., 2011; Ismail Mouzam, et al., 2011)*

Niosomes were designed by $3^2$ full factorial design to study the interaction variables of formulation on characterization of niosomes. Amount of polysobate 80 and cholesterol were selected as independent variable. Amount of Etoposide (10mg) were kept constant. The variables and the level of each factor were shown in table 7.1.

**Table 7.1:** Variables in $3^2$ full factorial design

| **Independent variable factors** | **Levels used** | | |
|---|---|---|---|
| | **Lower (-1)** | **Middle (0)** | **Upper (+1)** |
| Polysorbate 80 ($X_1$) mg | 5 | 10 | 15 |
| Cholesterol ($X_2$) mg | 5 | 10 | 15 |
| Dependent variable, response Y= Entrapment efficiency | | | |
| **Amount of drug used was 10 mg in all formulation** | | | |

Each row identifies an experiment and each row provides a result (response). The levels of factors studied were chosen that the irrelative difference was adequate to have a measurable effect on response, along with the information that the selected

levels are within practical use. The formulation of the factorial design is represented in table 7.2.

**Table 7.2:** Designing the formulation with a $3^2$ full factorial design

| S.NO | Code of formulation | Polysorbate 80 concentration ($X_1$) | Cholesterol concentration ($X_2$) |
|---|---|---|---|
| **1** | EN1 | -1 (5mg) | -1 (5mg) |
| **2** | EN2 | -1 (5mg) | 0 (10mg) |
| **3** | EN3 | -1 (5mg) | +1 (15mg) |
| **4** | EN4 | 0 (10mg) | -1 (5mg) |
| **5** | EN5 | 0 (10mg) | 0 (10mg) |
| **6** | EN6 | 0 (10mg) | +1 (15mg) |
| **7** | EN7 | +1 (15mg) | -1 (5mg) |
| **8** | EN8 | +1 (15mg) | 0 (10mg) |
| **9** | EN9 | +1 (15mg) | +1 (15mg) |

**PREPARATION OF NOVEL NIOSOMAL DELIVERY SYSTEM:**

*(Balasubramanian A., et al., 2002)*

The Novel niosomal delivery system was prepared by the modification of reverse phase evaporation technique [REV]. Polysorbate 80 and cholesterol were dissolved in a mixture of diethyl ether and chloroform.

Etoposide was dissolved in the lipid solution. The organic phase was the added to the aqueaes phase (phosphate buffer saline pH 7.4). The resulting two phase system was homogenized using a homogenize [REMI] for 3 minutes at 8000 rpm.

The organic phase was removed at 40ºC under reduced pressure. The reduced suspension was further homogenized for 1 min. The suspension was then heated on a water bath at 60ºC for 10 min to yield the niosomes.

The unentrapped was removed by centrifugation of dispersion at 14,000 rpm for 60 min in a centrifuge. The supernatant was removed and the pellet (residue) was resuspended in PBS pH 7.4.

# Evaluation Of Niosomes

# 8. EVALUATION OF NIOSOMES

## 8.1. Determination of niosomes recovery (%), entrapment efficiency (%EE) and drug loading efficiency (%LE):

*(Kumar Abhinav, et al., 2011)*

To determine niosome recovery, entrapment efficiency and drug loading efficiency, the niosomal aqueous suspension was ultracentrifuged at 3000 rpm for 5 min, supernatant was removed and sediment was washed twice with distilled water in order to remove the adsorbed drug.

The niosomal recovery (%) was calculated as:

$$\text{Niosomes recovery (\%)} = \frac{\text{Amount of niosomes recovered}}{\text{Amount of drug + excipients}} \text{ X } 100$$

The entrapment efficiency (%EE) was calculated using formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Amount of drug in niosomes}}{\text{Amount of drug used}} \text{ X } 100$$

The drug loading efficiency (%LE) was calculated as:

$$\text{Drug loading efficiency (\%)} = \frac{\text{Amount of drug in niosomes}}{\text{Amount of niosomes recovered}} \text{ X } 100$$

## 8.2. Shape and surface morphology: (*Vedha Hari B.N., 2011*)

The morphology of the noisome was studied by scanning electron microscopy (FEI Quanta FEG 200 HR- SEM). Samples were prepared by allowing them to air dry on an aluminum stub with double- sided adhesive tape. The stub was fixed into a

simple holder and placed in the vacuum chamber of scanning electron microscope and randomly scanned for surface morphology.

**8.3. Analysis of vesicle size and polydispersity index:**

*(Sandeep Kumar Sharma, et al., 2009; Ismail Mouzam, et al., 2011)*

The size analysis of niosomes was performed by laser scattering method. The z- average diameter of prepared vesicle was determined dynamic light scattering using a Zetasizer, (Zetasizer nano-zs ZEN3600; Malvern Instrument, Worcestershire, UK). For the measurement, 100µl of the formulation was diluted with an appropriate volume of PBS $p^H$ 7.4 and vesicle diameter and polydispersity index were determined.

**8.4. Determination of vesicle surface charge (Zeta potential):**

***(****Varaporn Buraphacheep Junyaprasert, et al., 2008)*

The charge on vesicles is expressed in terms of zeta potential. The vesicle surface charge can play an important role in the behavior of niosomes *in vivo* and *in vivo*. In general, charged niosomes are more stable against aggregation and fusion than uncharged vesicles. In order to obtain an estimate of the surface potential, the zeta potential of individual niosomes can be measured by Zetasizer. Zeta potential of niosomes was measured in a Zetasizer ZEN3600 (Malvern instruments, UK). The niosomes were dispersed in phosphate- buffered saline ($p^H$ 7.4), and the zeta potential was determined.

**8.5. Compatibility study:**

**8.5.1 Compatibility studies by Fourier transform infrared Spectroscopy (FTIR):**

*(Pandey shivanand, et al., 2010)*

FTIR study was carried out to check compatibility of drug with surfactant and cholesterol. The interaction between drug substance, surfactants and cholesterol of

best formulations were evaluated by comparing the IR spectrum of pure drug sample. Infrared spectrum of etoposide was determined on Fourier transform infrared spectrophotometer using potassium bromide dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug loaded noisome. Infrared (IR) spectra of the formulation were recorded between 400 and 4000 $cm^{-1}$. The spectra of drug and other ingredients in the formulations were compared with that of the original spectra.

**8.5.2. Compatibility studies by Differential scanning calorimetry (DSC):**

*(Y. Prasanna raju, et al., 2007)*

The interaction between drug substance, surfactants and cholesterol of best formulations were evaluated by comparing the IR spectrum of pure drug sample. Thermograms of the pure drug Etopside and drug loaded niosomes were analysed using a Differential scanning calorimeter (Shimadzu W70 thermal analyzer). The samples were heated in a hermetically sealed aluminum pans in the temperature range of 100-300ºC at heating rate of 10ºC /min under nitrogen flow of 30ml/min.

**8.6. *In-vitro* release studies:**

(*Anand Kumar Y., et al., 2010; Kandasamy Ruckmani, et al., 2010*)

In vitro release was studied using a dialysis bag (Dialysis membrane, 12,000 - 14,000 molecular weight cut off) as a donor compartment. Niosomes containing entrapped etoposide obtained after configuration of 2 ml of the formulation were resuspended in 1ml of PBS pH 7.4, and used for the release study. The dialysis membrane was soaked in warm water for 10 minutes, one end sealed with a clip, the

noisome preparation or plain etoposide solution was pipette into the bag and the bag was sealed with another closure clip for to prevent leakage. The dialysis bag was placed in 100 ml of PBS, pH 7.4, at 37 ± 2ºC. The medium, which acted as the receptor compartment, was stirred at 100 rpm. Samples of medium (5 ml) were withdrawn hourly and replaced with fresh buffer and etoposide absorbance at 284 nm was measured using PBS as blank. Results were the mean values of three runs.

Dialysis membrane

Dialysis bag


Magnetic stirrer with thermostat

**Figure 8.1:** Schematic assembly of In-vitro release study

**8.7. Kinetics of *In-vitro* drug release:**

*(Brahmankar D.M., 2006; Anupriya kapoor, et al., 2011)*

To study the release kinetics of *in-vitro* drug release, data was applied to kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas.

- **Zero order:**

$$\mathbf{C = K_0 t}$$

Where $K_0$ - is the zero-order rate constant expressed in units of concentration/time

t -is the time in hrs.

- **First order:**

$$\mathbf{LogC = LogC_0 - Kt / 2.303}$$

Where $C_0$ - is the initial concentration of drug,

*K* - is the first order constant

*t* - is the time in hrs.

- **Higuchi:**

$$\mathbf{Qt = Kt^{1/2}}$$

Where $Q_t$ - is the amount of the release drug in time t,

*K*- is the kinetic constant and

t- is time in hrs.

- **Korsmeyer Peppas:**

$$\mathbf{Mt / M\infty = Kt\ n}$$

Where $M_t$ - represents amount of the released drug at time t,

$M_\infty$- is the overall amount of the drug (whole dose) released after 12 hrs

K- is the diffusional characteristic of drug/ polymer system constant

n- is a diffusional exponent that characterizes the mechanism of release of drug.

The value of n indicates the drug release mechanism related to the geometrical shape of the delivery system, if the exponent n = 0.5, then the drug release mechanism is Fickian diffusion. If n < 0.5 the mechanism is quasi-Fickian diffusion, and 0.5 < n < 1.0, then it is non-Fickian or anomalous diffusion and when n = 1.0 mechanism is non Fickian case II diffusion, n> 1.0 mechanism is non Fickian super case II.

**8.8. Sterilization technique:**

The formulated Etoposide noisomes were sterilized by membrane filtration (Mechanical method) using membrane filter with pore size of 0.22 μm in an aseptic condition using laminar air flow. All glass containers were sterilized by autoclave, 115-120 ºC at 30 min (Moist heat Sterilization).

**8.8.1. Test for sterility:** *(IP, 2007)*

Sterility tests were based upon the principle that if bacteria or fungi are placed in a medium which provided nutritive material, moisture, and the desired pH and kept

at a favorable temperature, the organism will grow and their presence can be indicated by the growth in originally medium.

The tests for sterility were done by detecting the presence of viable forms of bacteria, fungi and yeast in or on preparations. The tests were carried out under strict aseptic techniques in order to avoid accidental contamination of the preparation.

**Sterility test for niosomes:**

The niosomes were subjected to sterility test. The test for sterility was intended for detecting the presence of viable forms of bacteria, fungi, and yeast in preparations. The tests were carried out under aseptic conditions to avoid contamination of the product during the test.

**Culture media:**

**For anaerobic bacteria and fungi:**

**Medium:** Soyabean casein medium.

This medium can be used for the detection of anaerobic bacteria and fungi.

**Preparation of soyabean casein medium:**

**Table 7.5:** Preparation of soyabean casein medium

| S.No. | Ingredients | Quantity |
|---|---|---|
| 1 | Pancreatic digest of casein | 17.0 g |
| 2 | Papic digest of soyabean | 3.0 g |
| 3 | Meat | 5.0 g |
| 4 | Sodium chloride | 2.5 g |
| 5 | Dibasic potassium phosphate | 2.5 g |
| 6 | Dextrose | 2.5 g |
| 7 | Distilled water | 1000 mL |

The above ingredients were dissolved completely in 1000 mL of distilled water and the medium was boiled for 10 minutes. The pH was adjusted to 7.3 ± 0.2. Media was distributed into 9 cm diameter petri dishes

**Sterilization of medium:**

Anaerobic culture medium was sterilized by autoclaving at a pressure of 15 lbs/in² and at a temperature of 121ºC for 15 minutes. Autoclaved medium should be kept in room temperature before inoculation of the sample.

**Incubation:**

The sterilized niosomal formulation were placed in to fluid thioglycollate medium and incubated at 20ºC - 25ºC for not less than seven days.

At intervals during the incubation period, the media were examined visually for microbial growth.

**Control test**

In order to support the above performed test, a negative control test was also carried out for quality control maintenance.

Negative control test was carried out in order to confirm that the media and the environment provided for incubation were suitable for the growth of micro organism.

**8.9. In vivo drug targeting studies:**

*(Lakshmi P.K. and Shyamala Bhaskaran, 2009; Doijad R.C., et al., 2008)*

To perform this animal studies, protocol was submitted and received permission from Institutional Animal Ethics Committee (Approval No. 409/01/a CPCSEA dated on 22.07.2011).

Best formulation was selected on the basis of In-vitro release study. Selected formulation was sterilized in UV radiations. Sterilized niosomal formulation was employed for In-vivo tissue distribution study.

Albino mice are used for this test because of uniform breed as the result the variability factor in the experimentation is reduced, economic and easily available. Also, using mice is cost effective.

To perform this study, the followings were selected,

**Animal:** Mice

**Species:** Albino

**Gender:** Either sex

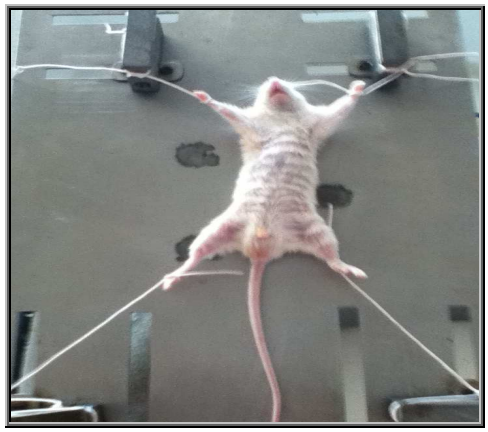
**Number of animals:** Ten

**Weight:** 20-25 g

Ten healthy mice weighing 20-25gms were selected. The animals were fasted for 12 hours by maintaining a constant day and night cycle. Mice of either sex were divided into two groups each containing five mice. After redispersing the Etoposide loaded niosomes in phosphate buffer saline (pH 7.4) were administered intravenously in the tail vein of group I and the plain Etoposide were given intravenously to group II at a dose of 1.3mg/kg.

The mice were sacrificed after 12 hours by giving anesthetic ether and the organs like liver, lungs, spleen, kidney and brain was isolated, homogenized separately and centrifuged at 15,000 rpm to obtain the supernatant. Further it was filtered through a membrane filter with pore size of 0.22 μm. The drug content was estimated using UV- spectrophotometer at 284nm.

Tail vein injection



Sacrificed after 12 hours

Mice skin were cut opened

Isolation of brain

**Figure 8.2:** Sequence of process involved in isolation of organs

Secluded Liver

Secluded Spleen

Secluded Kidney

Secluded Lungs

Secluded Brain

**Figure 8.3:** Collective display of isolated organs

**8.10. Stability studies:** *(Manavalan R and Ramasamy S., 2004)*

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods (FDA, 1998).

Stability of pharmaceutical preparation can be defined as the capability of particular formulation (dosage form) in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicology specifications throughout its shelf life.

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. Generally, the observation of the rate at which the product degrades under normal room temperature requires a long time. To avoid this undesirable delay, the principles of accelerated stability studies are adopted. The International Conference on Harmonization (ICH) Guidelines titled "Stability testing of New Drug Substances and Products" describes the stability test requirements for drug registration application in the European Union, Japan and the States of America.

ICH specifies the length of study and storage conditions

- **Long-Term Testing**: 25°C ± 2°C at 60% RH ± 5% for 12 Months
- **Accelerated Testing:** 40°C ±2°C at 75% RH ± 5% for 6 Months

**Procedure:**

*(Doijad R.C., et al., 2008; Pandey Shivanand., et al., 2010)*

The optimized formulation EN5 was subjected to stability testing upto 3 months at refrigerator temperature, room temperature and elevated condition ($4^0$ C $\pm 1^0$ C , $25^0 \pm 2^0$ C at 60% RH±5% RH and $40^0$ C $\pm 2^0$ C at 75% RH ±5% RH ). The samples were withdrawn after periods of 1st month, 2nd month and 3rd month. The samples were analyzed for its parameters such as,

- Percentage drug retained
- *In-vitro* drug release
- Test for sterility

.

# Results

# And

# Discussion

# 9. RESULTS AND DISCUSSION

## 9.1. Identification of Drug:

### 9.1.1. Organoleptic Properties:

Colour : white

Odour : Odorless

Appearance : Crystalline powder

### 9.1.2. Melting Point:

Melting point of Etoposide was found to be 250.6 ºC. The official melting point range for Etoposide is between 236-251°C. Hence, results were complied the limits specified in official Book.

### 9.1.3. Solubility Study:

**Table 9.1:** The solubility of Etoposide in various solvents

| S.No | Name of solvent | Solubility |
|---|---|---|
| 1. | Water | Sparingly soluble |
| 2. | 0.1NHCl | Sparingly soluble |
| 3. | Diethyl ether | Sparingly soluble |
| 4. | Ethanol | Slightly soluble |
| 5. | Methanol | Very soluble |
| 6. | Chloroform | Very soluble |

### 9.1.4. FTIR spectroscopy:

The FTIR spectrum of Etoposide was shown in Figure 9.1 and the interpretations of IR frequencies were showed in Table 9.2.

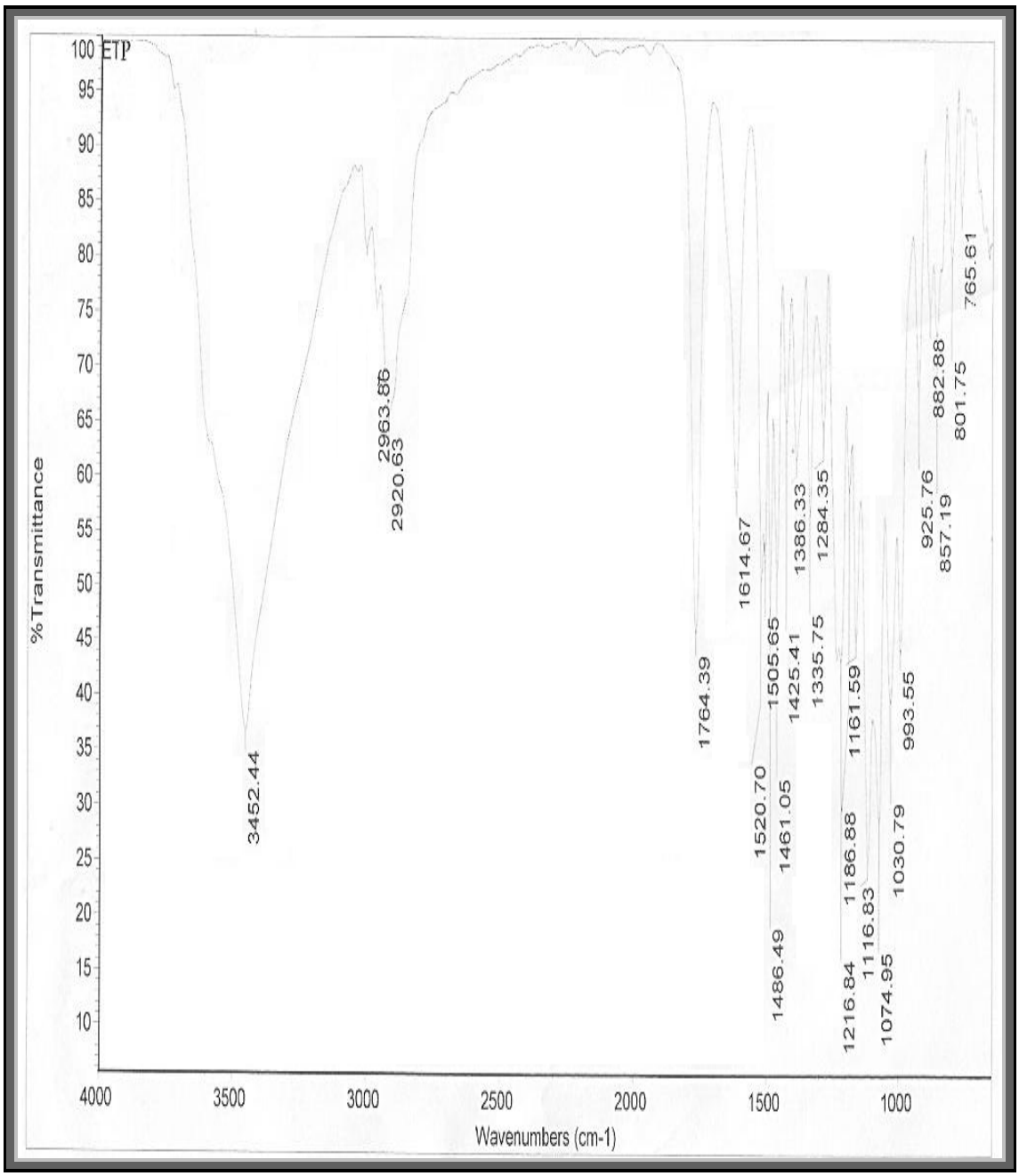



**Fig.9.1:** FTIR spectrum of Etoposide

**Table 9.2:** Characteristic Frequencies in FTIR spectrum of Etoposide

| S.No. | Transition | IR Range ($cm^{-1}$) | Absorption wave number of Etoposide ($cm^{-1}$) |
|---|---|---|---|
| 1 | O-H stretching | 3400 – 3200 | 3452.44 |
| 2 | $CH_3$ stretching | 2930 – 2920 | 2920.63 |
| 3 | C=O stretching | 1780 - 1760 | 1763.39 |
| 4 | C=C stretching | 1660-1610 | 1614.67 |
| 5 | C-C stretching | 1580-1500 | 1505.65 |
| 6 | COO stretching | 1430-1300 | 1425.1 |
| 7 | O-H stretching | 1380-1280 | 1335.75 |
| 8 | C-O stretching | 1300-1200 | 1284.35 |
| 9 | C-O-C stretching | 1150-1100 | 1116.83 |
| 10 | C-O stretching | 1075-1190 | 1074.95 |
| 11 | C-H bending | 995-985 | 993.55 |
| 12 | C-H bending | 860-900 | 882.88 |
| 13 | C-C bending | 680-725 | 682 |

**Interpretation of FTIR Spectrum:**

Major functional groups present in Etoposide show characteristic peaks in IR spectrum. Table 9.1 shows peaks observed at different wave numbers and the functional group associated with these peaks. The major peaks are identical to functional group of Etoposide. Hence, the sample was confirmed as Etoposide.

**9.1.5. UV Spectrophotometric Study:**

- The absorption maximum for Etoposide in methanol was found to be 285 nm

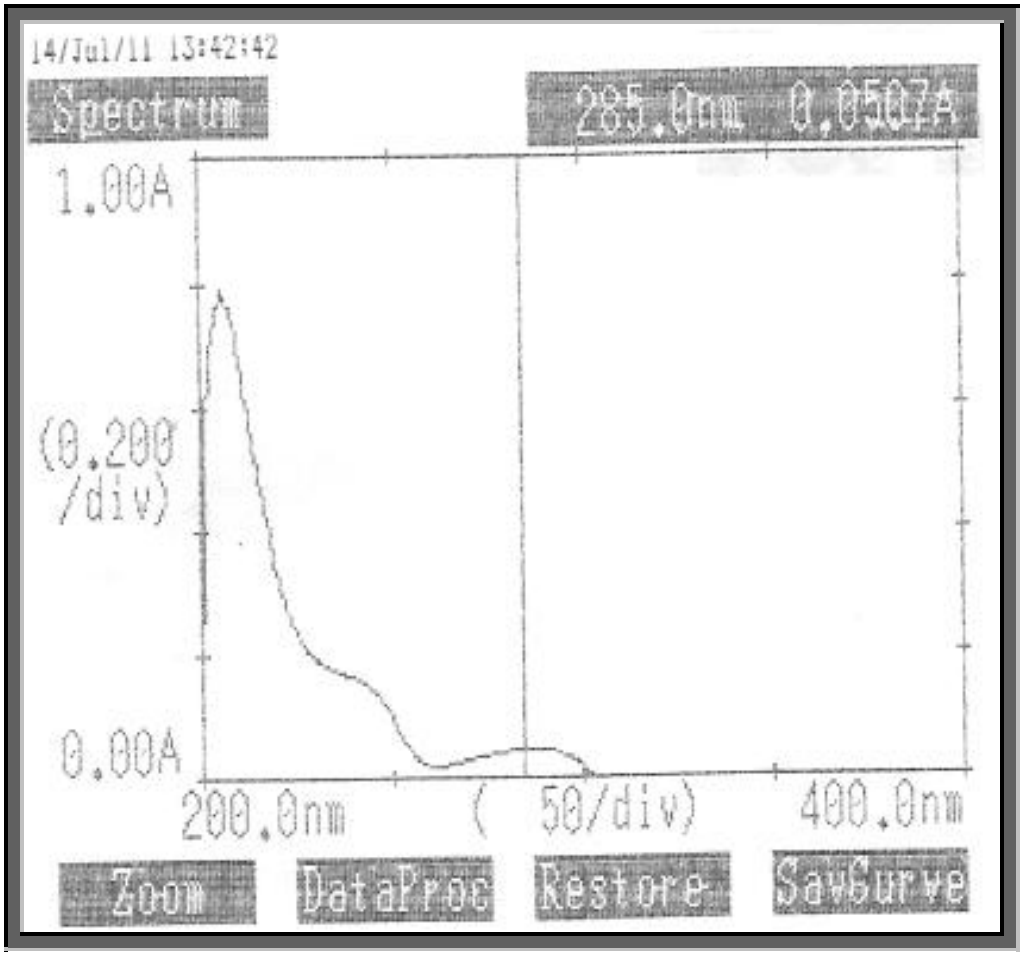



**Fig. 9.2:** $\lambda_{max}$ of Etoposide in methanol

❖ The absorption maximum for Etoposide in PBS pH 7.4 was found to be 284 nm

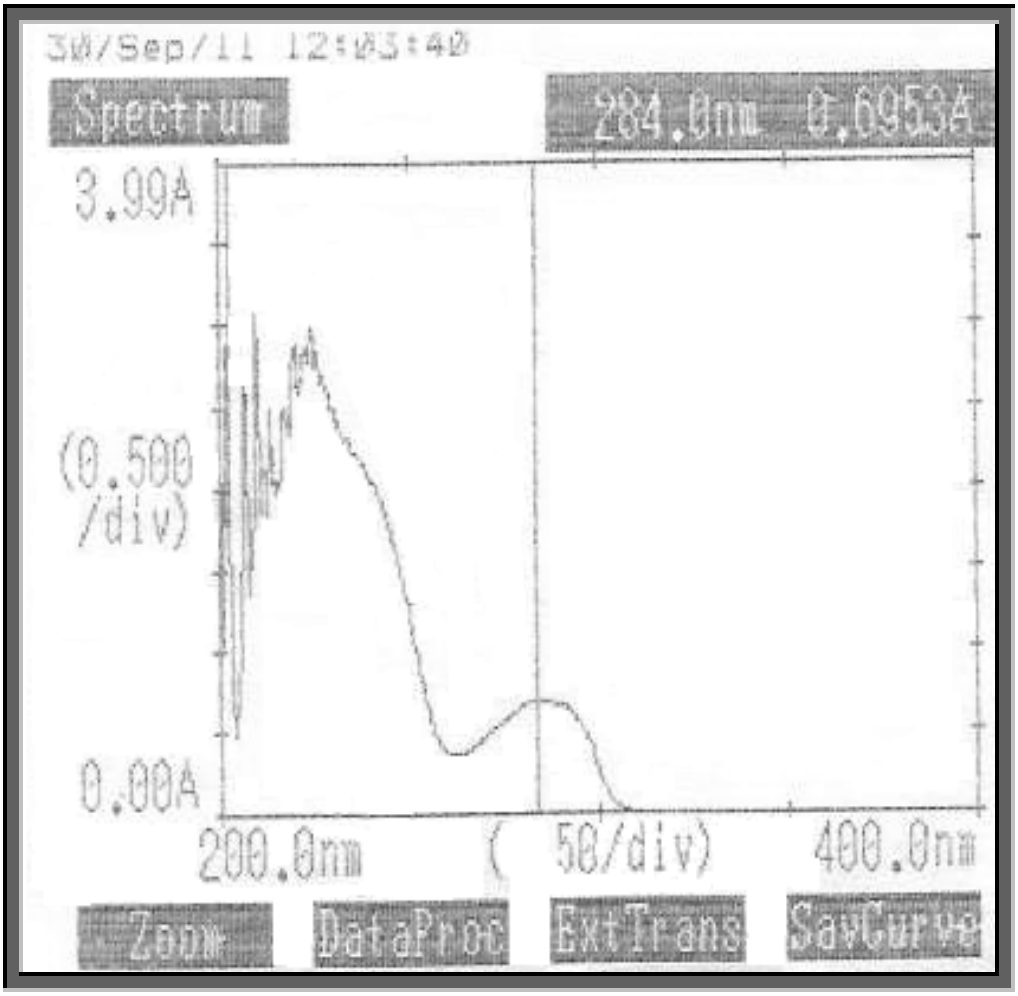



**Fig. 9.3:** $\lambda_{max}$ of Etoposide in PBS pH 7.4

**9.1.6. Calibration curve of Etoposide in methanol:**

UV absorption spectrum of Etoposide in methanol showed $\lambda_{max}$ at 285 nm. Absorbance obtained for various concentrations of etoposide in methanol were given in Table 9.3. The graph of absorbance vs concentration for Etoposide was found to be linear in the concentration range of 20-100 µg /mL. The drug obeys Beer- Lambert's law in the range of 20-100 µg /mL.

**Table 9.3:** Data of concentration and absorbance for Etoposide in methanol.

| S.No. | Concentration (µg/mL) | Absorbance |
|---|---|---|
| 1 | 0 | 0.0000 |
| 2 | 20 | 0.139 |
| 3 | 40 | 0.275 |
| 4 | 60 | 0.416 |
| 5 | 80 | 0.561 |
| 6 | 100 | 0.714 |

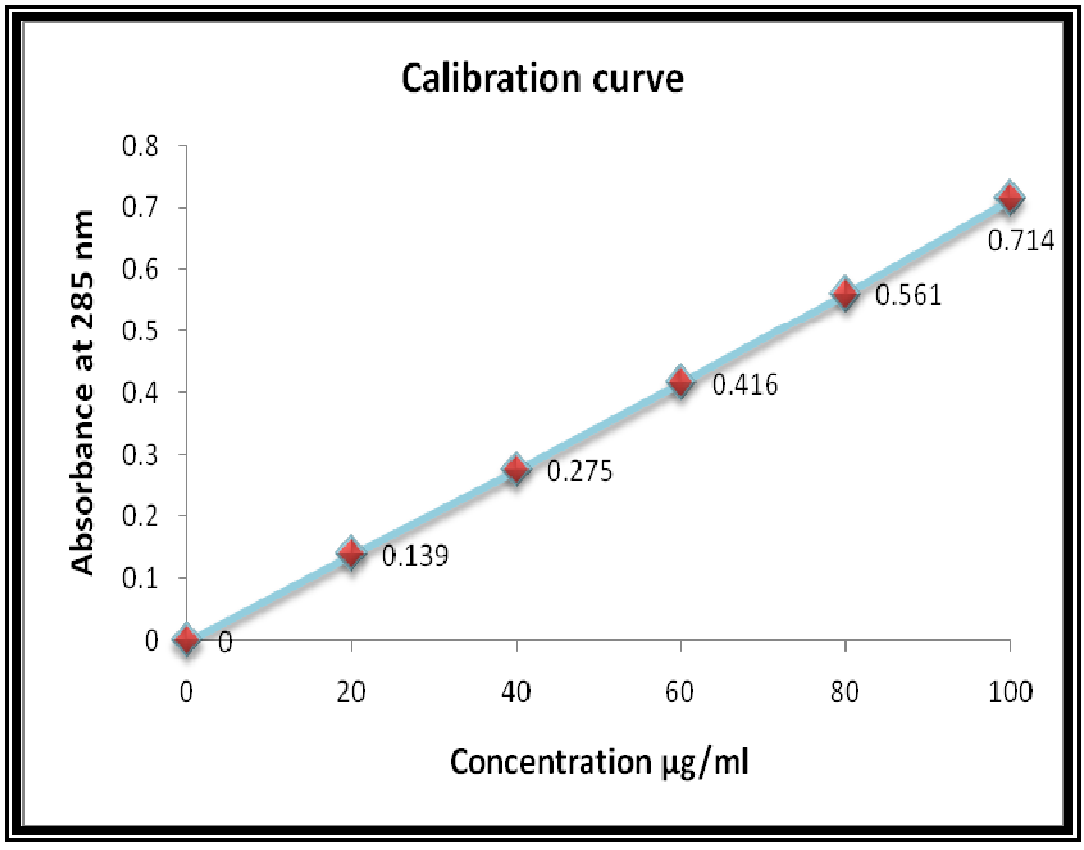



**Fig. 9.4:** Standard graph of Etoposide in methanol

**Table 9.4:** Data for Calibration Curve Parameters

| S.No. | Parameters | Values |
|---|---|---|
| 1 | Correlation coefficient (r) | 0.9997 |
| 2 | Slope (m) | 0.0071 |
| 3 | Intercept (c) | -0.0098 |

### 9.1.7. Calibration Curve of Etoposide in PBS $P^H$ 7.4:

UV absorption spectrum of Etoposide in PBS pH 7.4 showed $\lambda_{max}$ at 284 nm. Absorbance obtained for various concentrations of etoposide in methanol were given in Table 9.5. The graph of absorbance vs concentration for etoposide was found to be linear in the concentration range of 20-100 μg /mL. The drug obeys Beer- Lambert's law in the range of 20-100 μg /mL.

**Table 9.5:** Data of concentration and absorbance for Etoposide in PBS pH 7.4

| S.No. | Concentration (μg/mL) | Absorbance |
|---|---|---|
| 1 | 0 | 0.0000 |
| 2 | 20 | 0.165 |
| 3 | 40 | 0.318 |
| 4 | 60 | 0.486 |
| 5 | 80 | 0.649 |
| 6 | 100 | 0.798 |

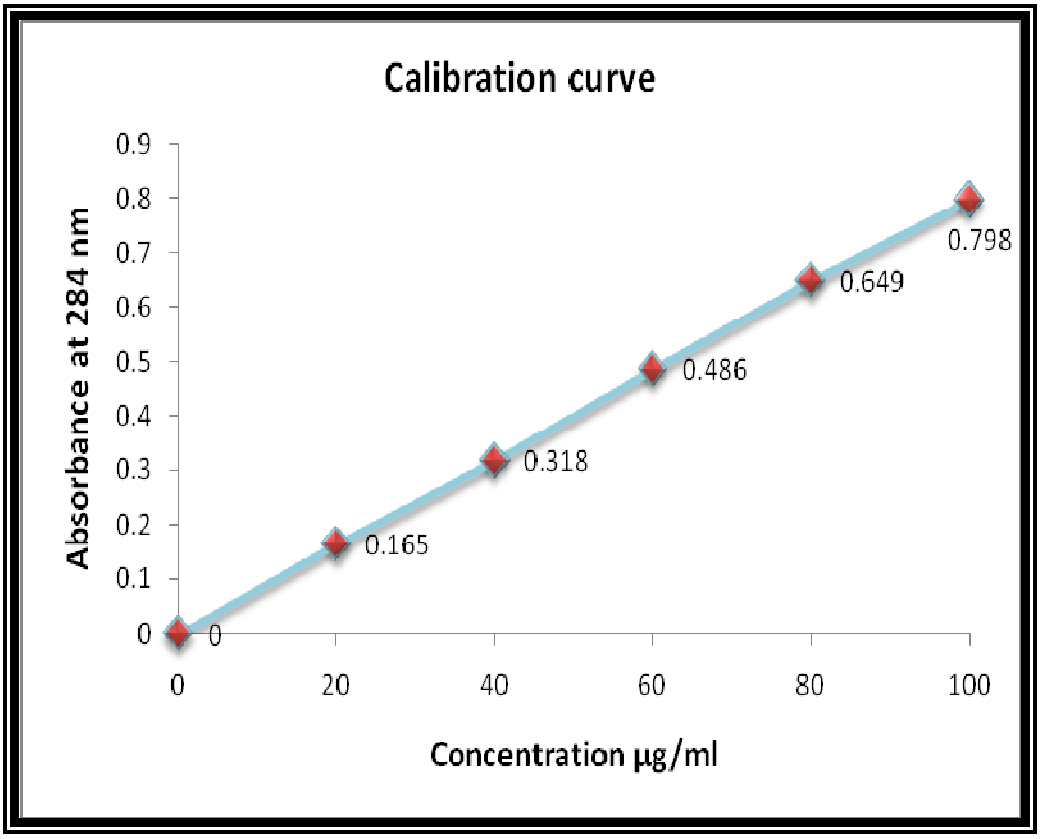



**Fig. 9.5:** Standard graph of Etoposide in PBS pH 7.4.

**Table 9.6:** Data for Calibration Curve Parameters

| S.No. | Parameters | Values |
|---|---|---|
| 1 | Correlation coefficient (r) | 0.9998 |
| 2 | Slope (m) | 0.00798 |
| 3 | Intercept (c) | 0.0041 |

### 9.1.8. Assay of Etoposide:

The percentage purity of drug was calculated by using calibration graph method. The values were recorded in the table 9.7.

**Table 9.7:** Assay of Etoposide

| S.No. | Percentage purity (%) | Average percentage purity (%) |
|---|---|---|
| 1 | 98.60 | 99.15±0.584 |
| 2 | 99.07 | |
| 3 | 99.77 | |

The official percentage purity of Etoposide is not less than 95% and not more than 105%. So, it can be declared as pure drug. The percentage purity of raw material Etoposide was found to be 99.15±0.584. Hence, the sample declared as pure.

**9.2. Evaluation of Niosomes:**

**9.2.1. Determination of niosomes recovery (%), entrapment efficiency (%EE) and drug loading efficiency (%LE):**

The results were shown in table 9.8 and figure.9.6. It is revealed that the highest **%** Recovery, %EE and %LE in the niosomes preparation were obtained.

The entrapment efficiency of nine batches of etoposide niosomes are recorded in Table 9.8. As the cholesterol concentration was increased the encapsulation efficiency was increased. The result indicates the cholesterol concentration plays a major role in drug entrapment efficiency rather than the surfactant concentration. The maximum entrapment efficiency was found in EN5 with 92.1%. The preparation parameters, such as cholesterol and surfactant concentrations were modified to obtain niosomes with higher entrapment efficiency.

**Table 9.8:** % Recovery, % EE and % LE of Etoposide niosomes

| S. No | Formulation code | Parameter | | |
|---|---|---|---|---|
| | | Niosomes recovery (%) | Entrapment efficiency (%) | Loading efficiency (%) |
| 1. | EN1 | 69.5 | 63.2 | 45.32 |
| 2. | EN2 | 63.20 | 71.1 | 44.93 |
| 3. | EN3 | 65.33 | 86.2 | 43.88 |
| 4. | EN4 | 58.36 | 65.0 | 44.52 |
| 5. | EN5 | 67.66 | 92.1 | 45.32 |
| 6. | EN6 | 68.51 | 84.01 | 35.08 |
| 7. | EN7 | 59.36 | 63.2 | 35.37 |
| 8. | EN8 | 66.31 | 75.02 | 32.22 |
| 9. | EN9 | 68.25 | 90.1 | 32.97 |

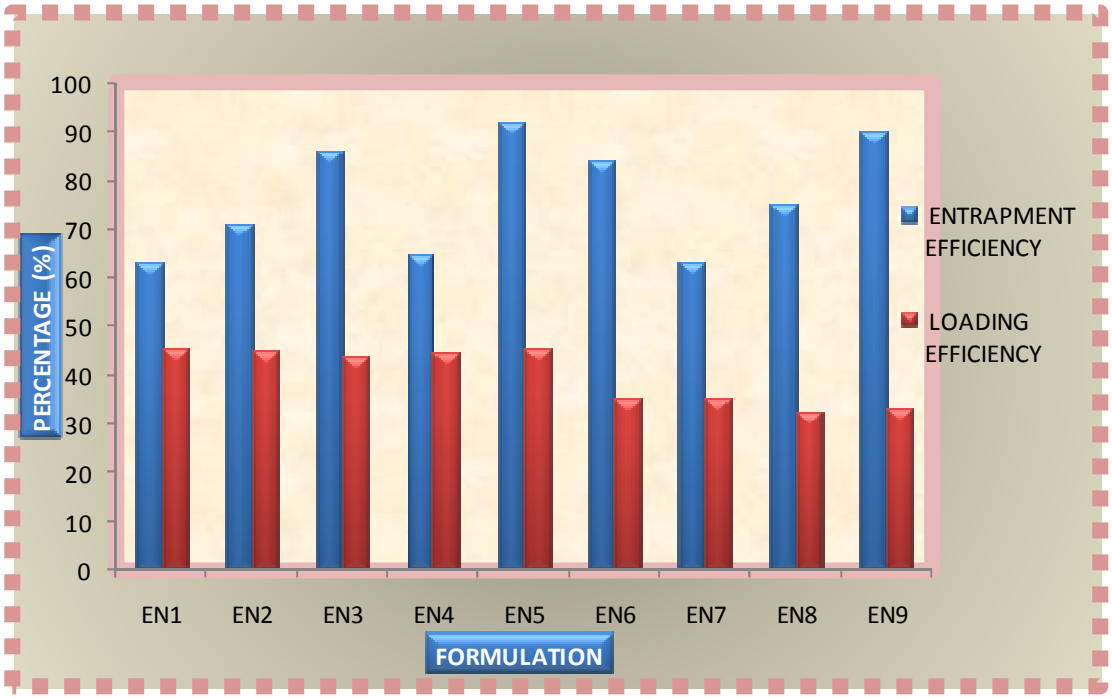



**Fig.9.6:** Comparison of entrapment efficiency and loading efficiency

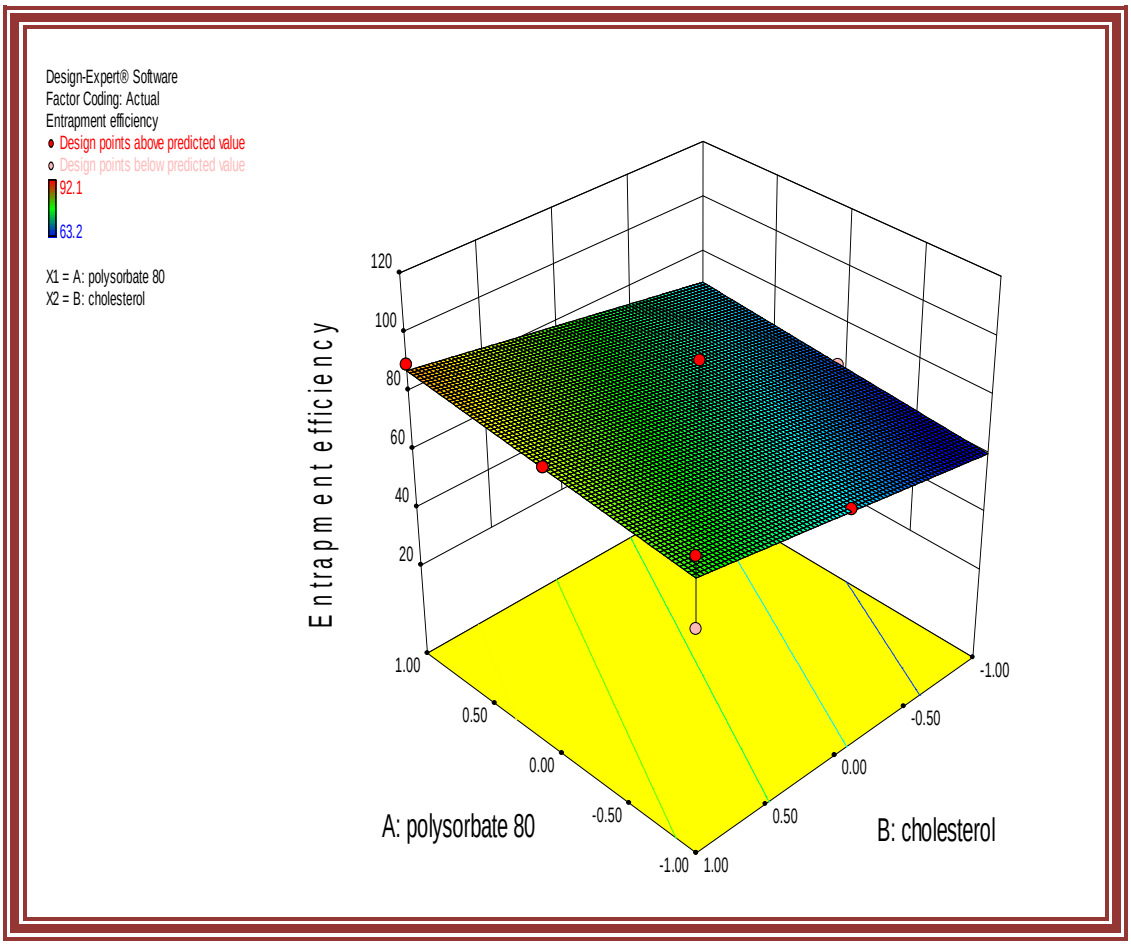
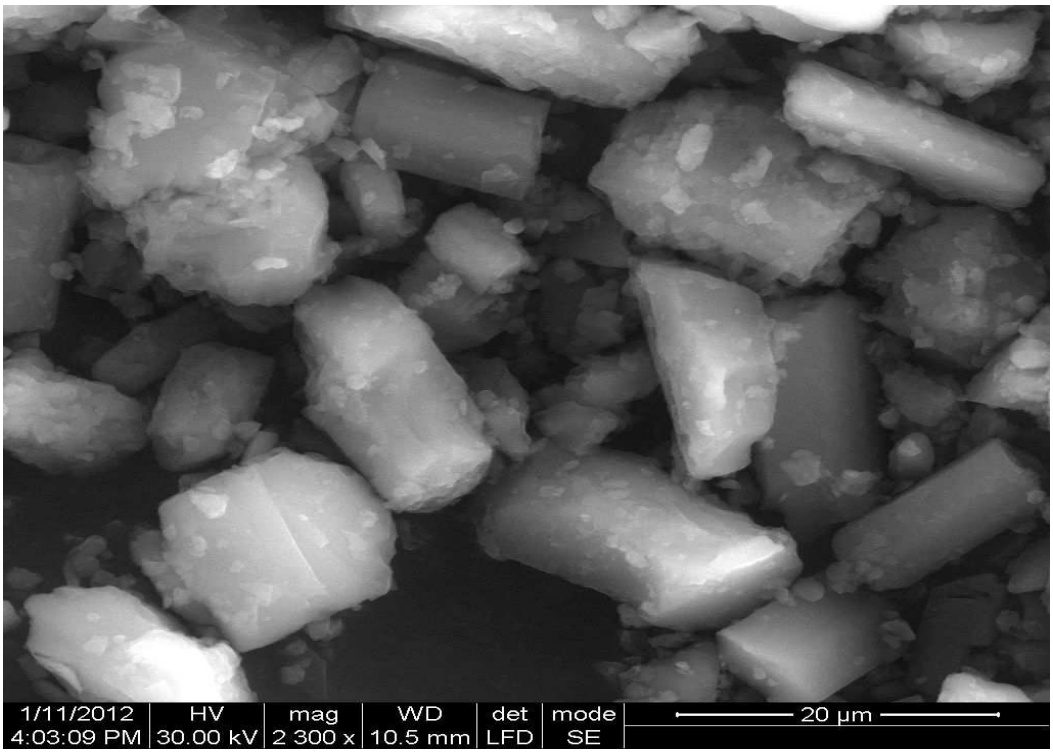



**Fig. 9.7:** Quadratic 3D surface plot showing the effect of Polysorbate 80 and cholesterol on Entrapment efficiency

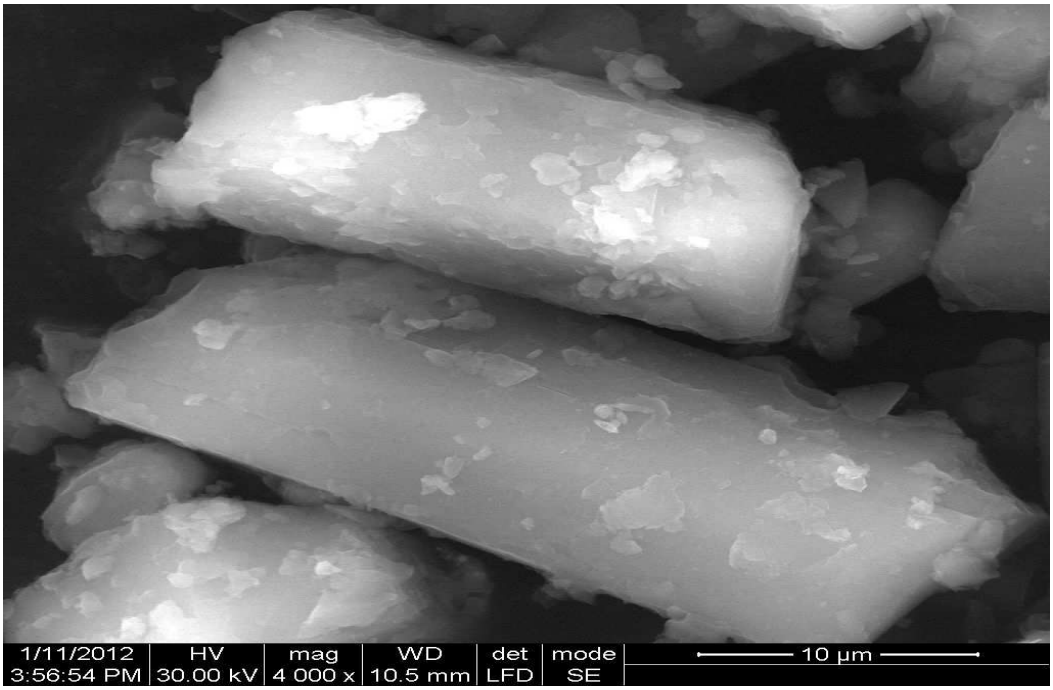
Entrapment efficiency was found to be a function of equimolar concentration of X1(polysorbate 80) and X2(cholesterol) respectively Fig. 9.7.

### 9.2.2. Shape and surface morphology:

Shape and surface morphology of niosomes was studied by Scanning Electron Microscopy (SEM) (FEI Quanta FEG 200 HR- SEM). SEM photographs of all formulations were shown in Fig. 9.8, 9.9, 9.10 and 9.11.

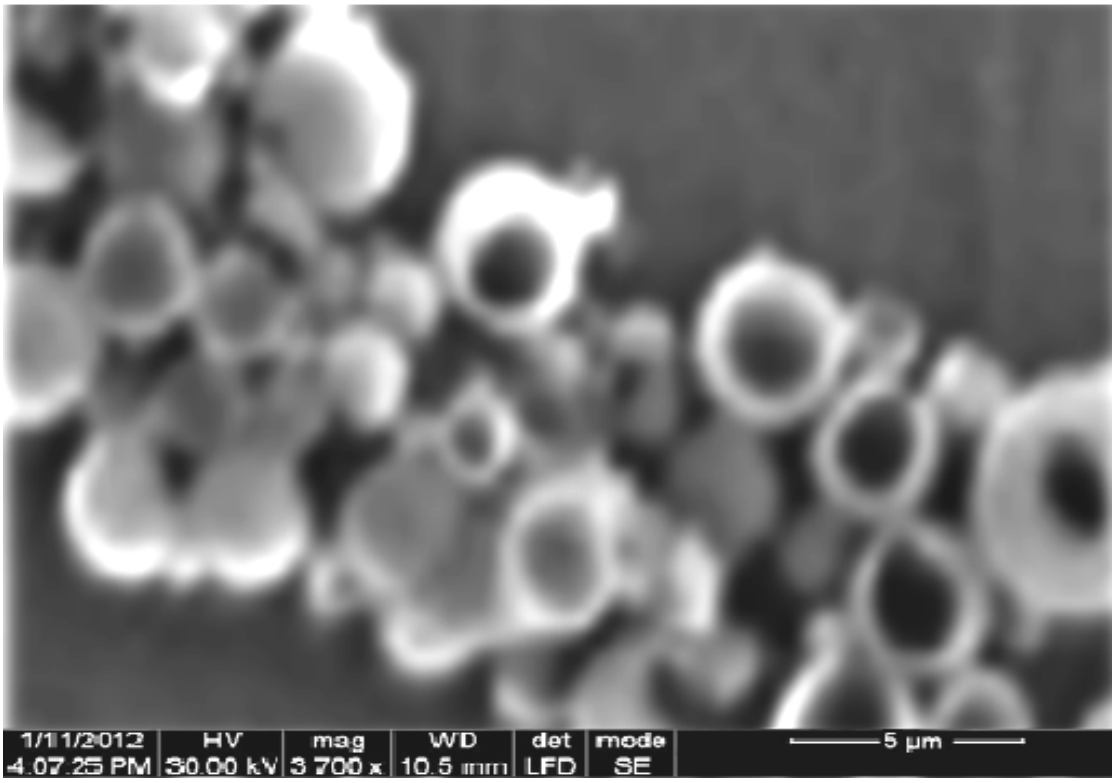



a)

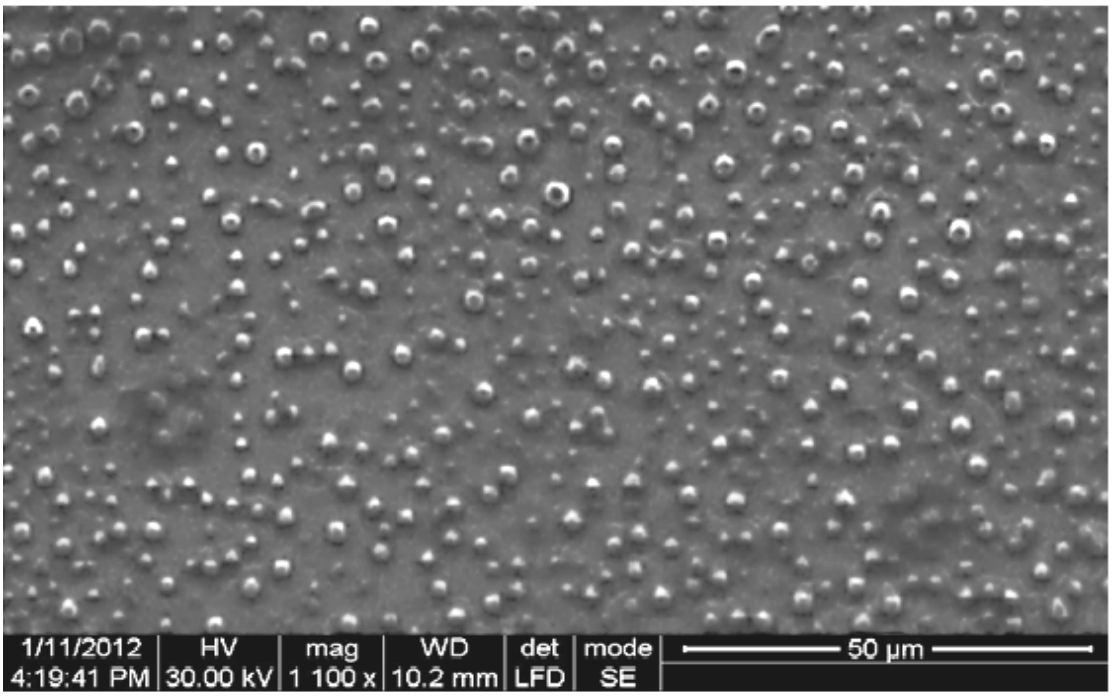



b)

**Fig.9.8:** SEM images of pure Etoposide

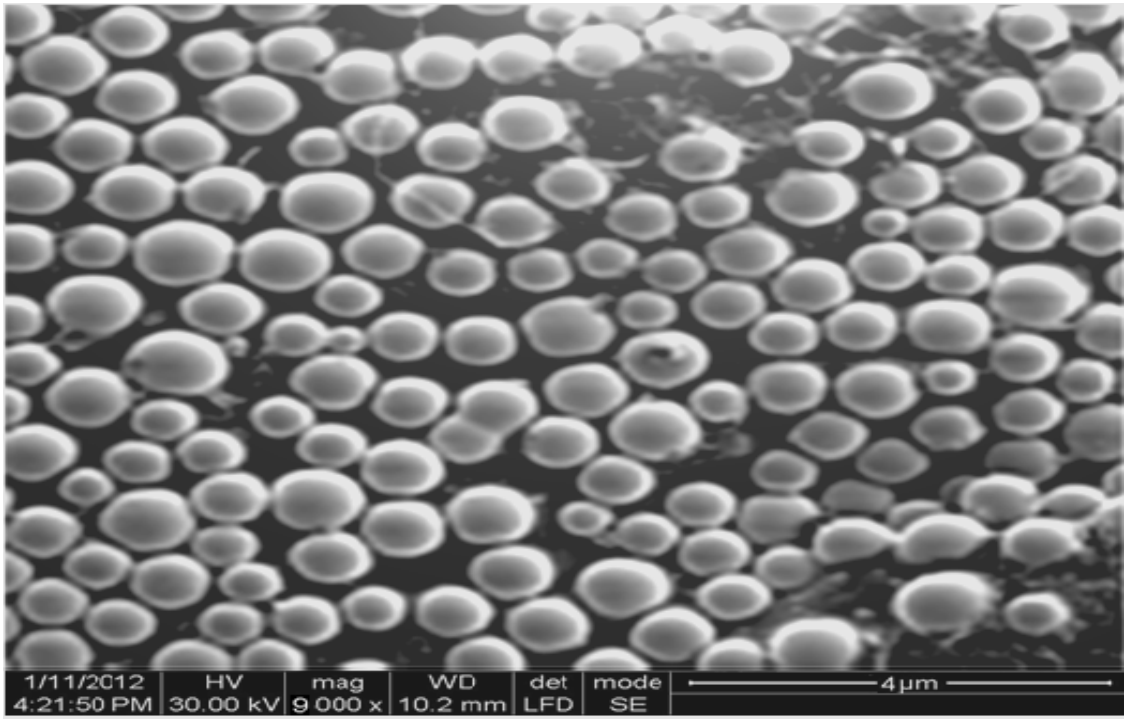



a)

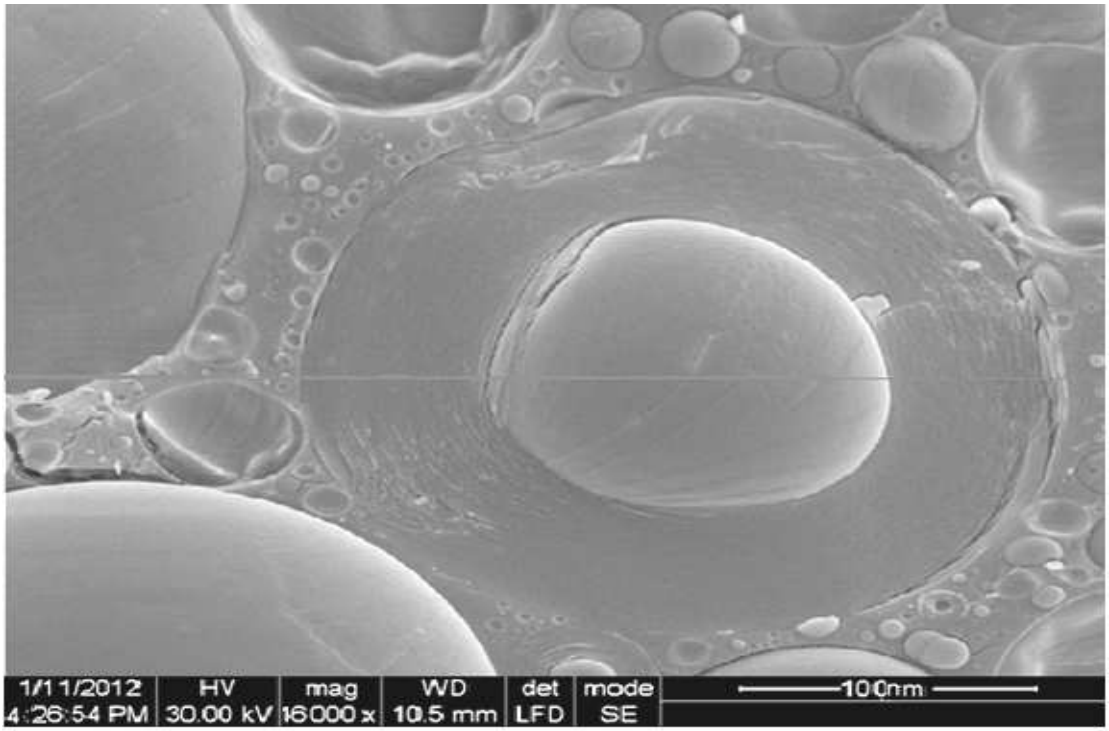



b)

**Fig.9.9:** SEM images of Formulation EN1

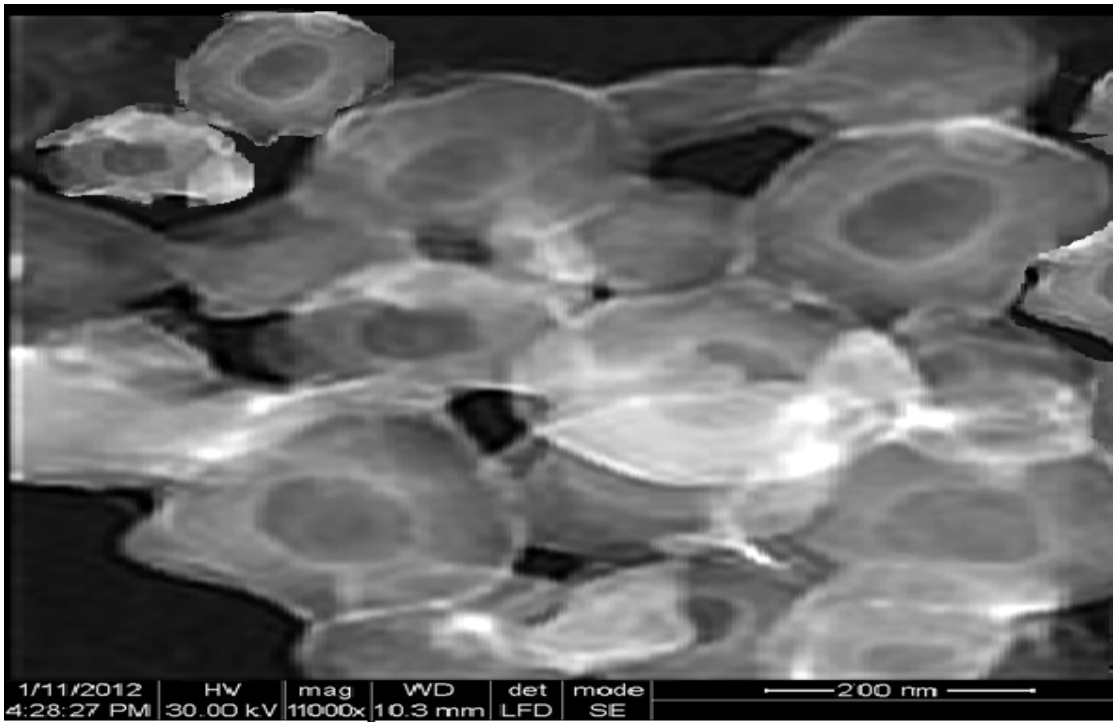



a)

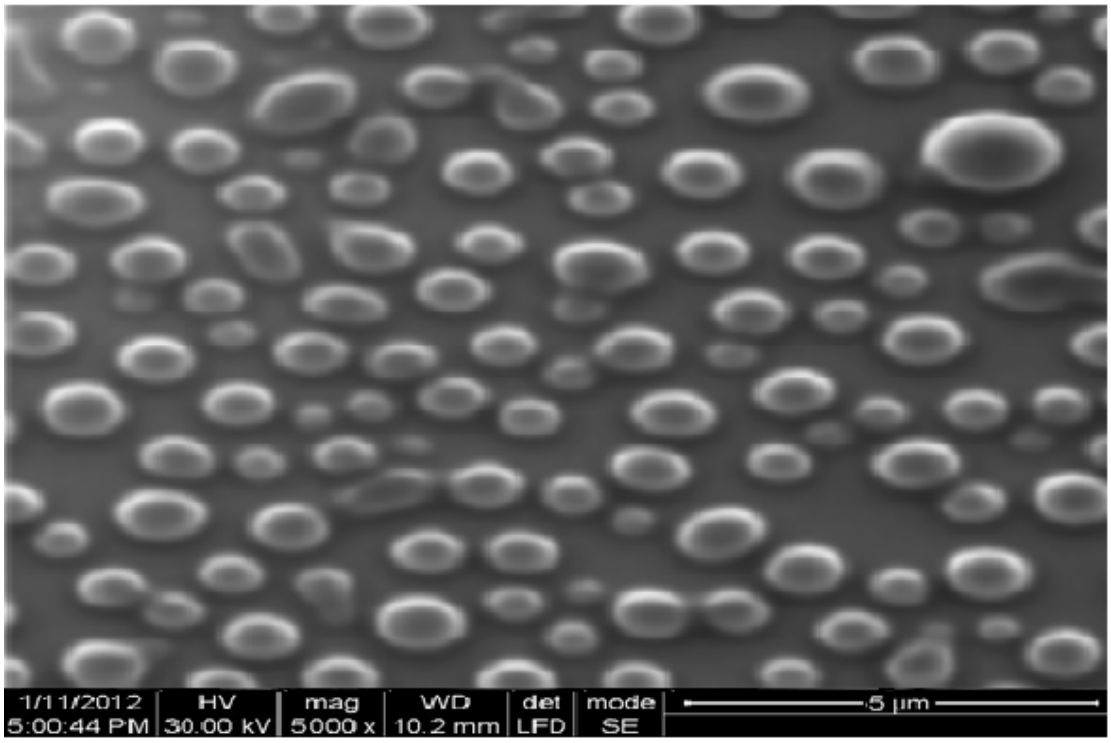



b)

**Fig.9.10:** SEM images of formulation EN5




a)




b)

**Fig.9.11:** SEM images of Formulation EN9

Etoposide niosomes have shown smooth and spherical shape with different sizes depending on the ratios of the surfactant and cholesterol used. The bilayered structure of the prepared niosomes that exist in disperse or in aggregate collections.

The photomicrography of pure Etoposide was shown in Fig. 9.8, this showed the presence of free crystals in the sample, while the SEM images of Formulated noisome showed bilayered structure, spherical shape, smooth surfaces and has no evidence of collapsed particles. Smooth surface reveals complete removal of solvent from the formulated niosomes and is the indication of good quality

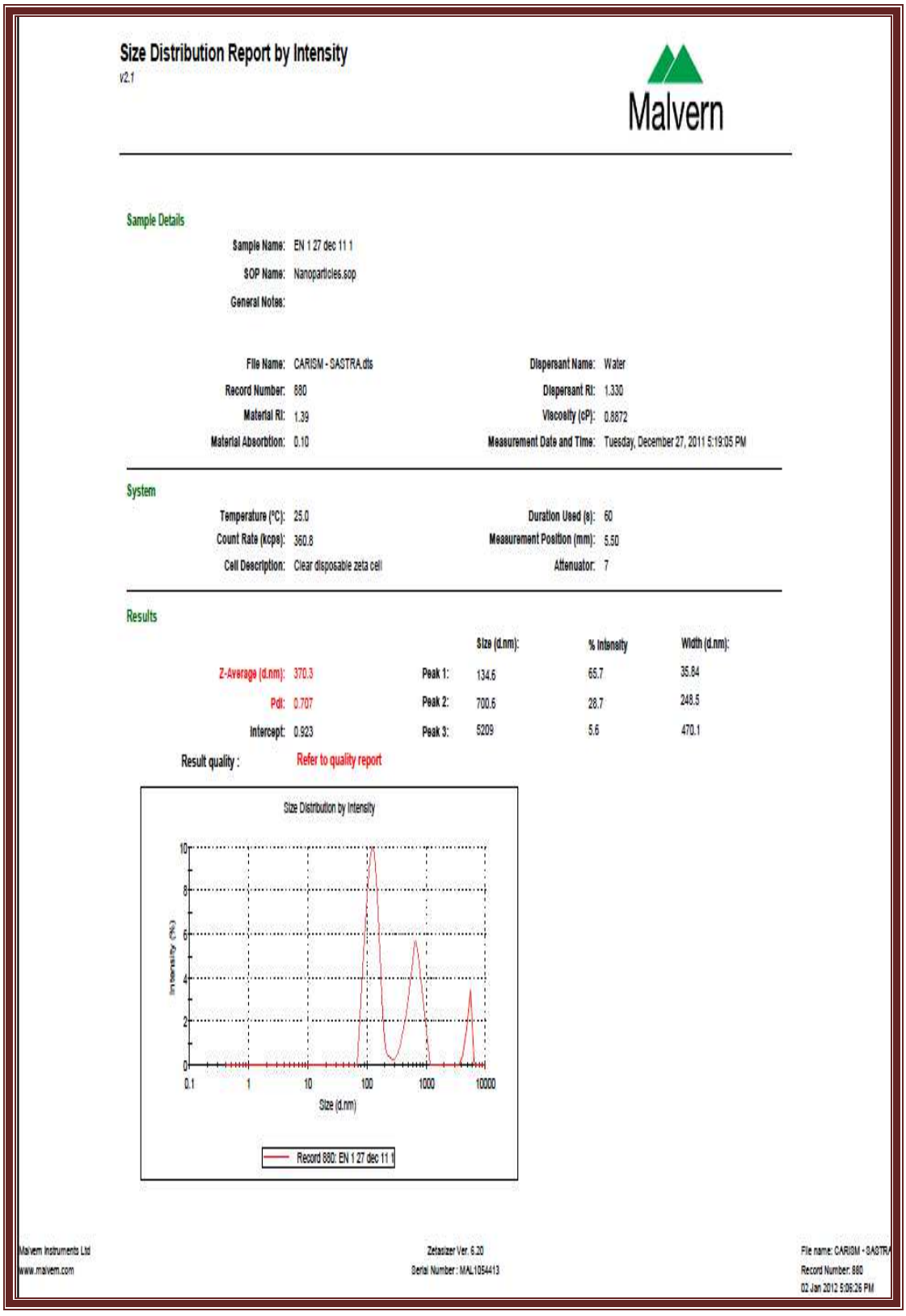
**9.2.3. Analysis of vesicle size and polydispersity index:**

The size distribution reports were shown in Fig. 9.12, 9.13 and 9.14. The size distributions along the mean diameter of the niosomes were measured by Dynamic Light Scattering Particle Size Analyzer (Malvern instruments).

Particles of all formulations were in nanosize having smooth spherical surface. For formulations EN1, EN5 and EN9 the particle size was found to be 134.6 nm, 131.4 nm and 136.7 nm respectively. As the surfactant concentration increased there was a decrease in particle size may be because of stabilizing effect of the surfactant, thereby preventing the aggregation of particles.

The particle size of niosomes composed of polysorbate 80 and cholesterol formulation EN5 in a 1:1 equimolar ratios were 131.4 nm, which was the least when compared to other formulations.

The particle size data showed that niosomes produced were of nanosize and had low polydispersity index which indicates relatively narrow particle size distribution for all preparations. The polydispersity index (PI) of formulations EN1, EN5 and EN9 was found to be 0.707PI, 0.522PI and 0.656PI.



Size Distribution Report by Intensity
v2.1

Malvern

Sample Details

Sample Name: EN 1 27 dec 11 1
SOP Name: Nanoparticles.sop
General Notes:

File Name: CARISM - SASTRA.dts
Record Number: 880
Material RI: 1.39
Material Absorbtion: 0.10
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Tuesday, December 27, 2011 5:19:05 PM

System

Temperature (°C): 25.0
Count Rate (kcps): 360.8
Cell Description: Clear disposable zeta cell
Duration Used (s): 60
Measurement Position (mm): 5.50
Attenuator: 7

Results

| | | | Size (d.nm): | % Intensity | Width (d.nm): |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 370.3 | Peak 1: | 134.6 | 65.7 | 35.84 |
| PdI: | 0.707 | Peak 2: | 700.6 | 28.7 | 248.5 |
| Intercept: | 0.923 | Peak 3: | 5209 | 5.6 | 470.1 |

Result quality : Refer to quality report

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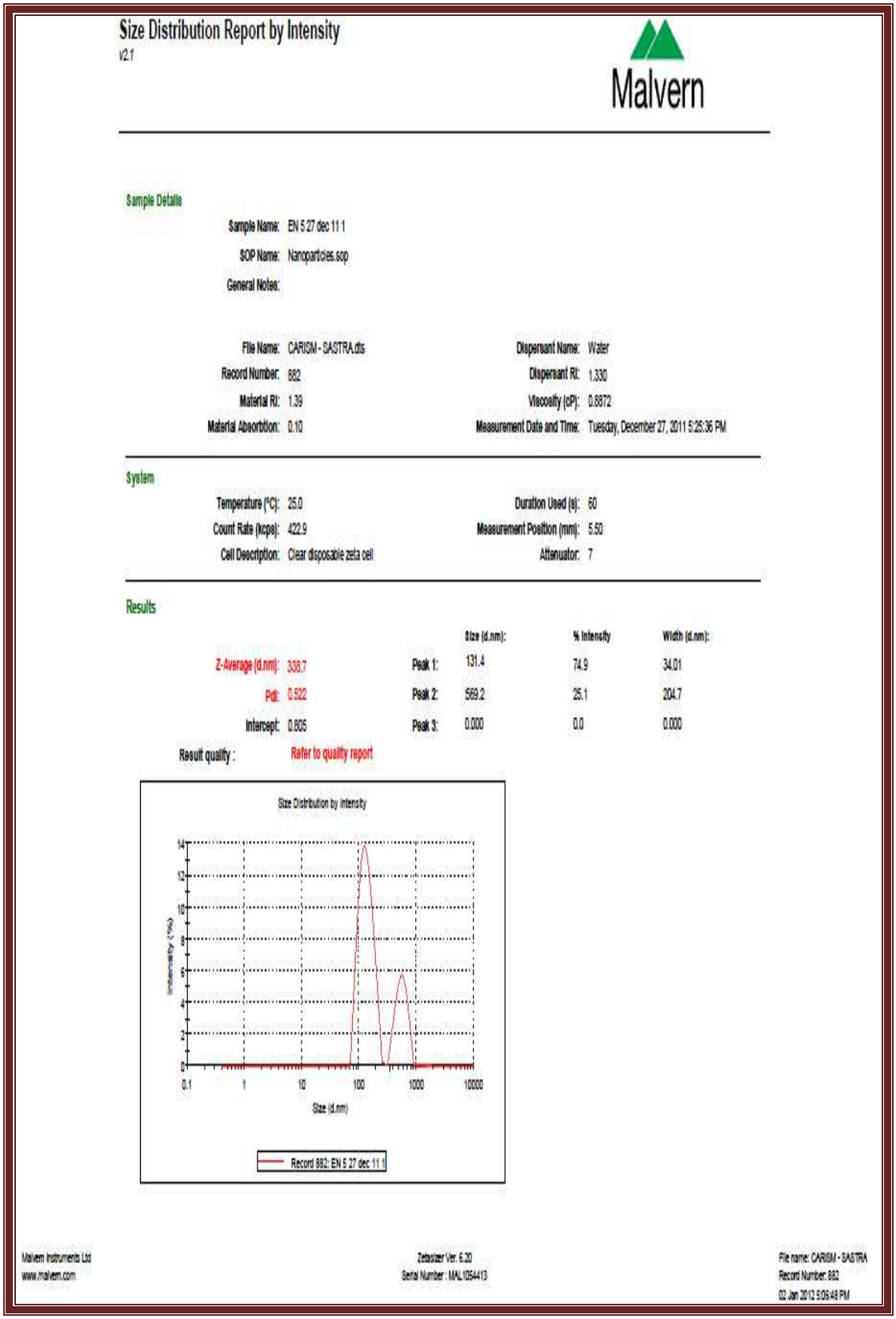
Serial Number : MAL1054413
File name: CARISM - SASTRA
Record Number: 880
02 Jan 2012 5:06:26 PM

**Fig.9.12:** Particle size distribution of Formulation EN1



Size Distribution Report by Intensity
v2.1

Malvern

Sample Details

Sample Name: EN 5 27 dec 11 1
SOP Name: Nanoparticles.sop
General Notes:

File Name: CARISM - SASTRA.dts
Record Number: 882
Material RI: 1.39
Material Absorption: 0.10
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Tuesday, December 27, 2011 5:25:36 PM

System

Temperature (°C): 25.0
Count Rate (kcps): 422.9
Cell Description: Clear disposable zeta cell
Duration Used (s): 60
Measurement Position (mm): 5.50
Attenuator: 7

Results

| | | | Size (d.nm): | % Intensity | Width (d.nm): |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 338.7 | Peak 1: | 131.4 | 74.9 | 34.01 |
| PdI: | 0.522 | Peak 2: | 569.2 | 25.1 | 204.7 |
| Intercept: | 0.805 | Peak 3: | 0.000 | 0.0 | 0.000 |

Result quality : Refer to quality report

Size Distribution by Intensity

Intensity (%)

Size (d.nm)

Record 882: EN 5 27 dec 11 1

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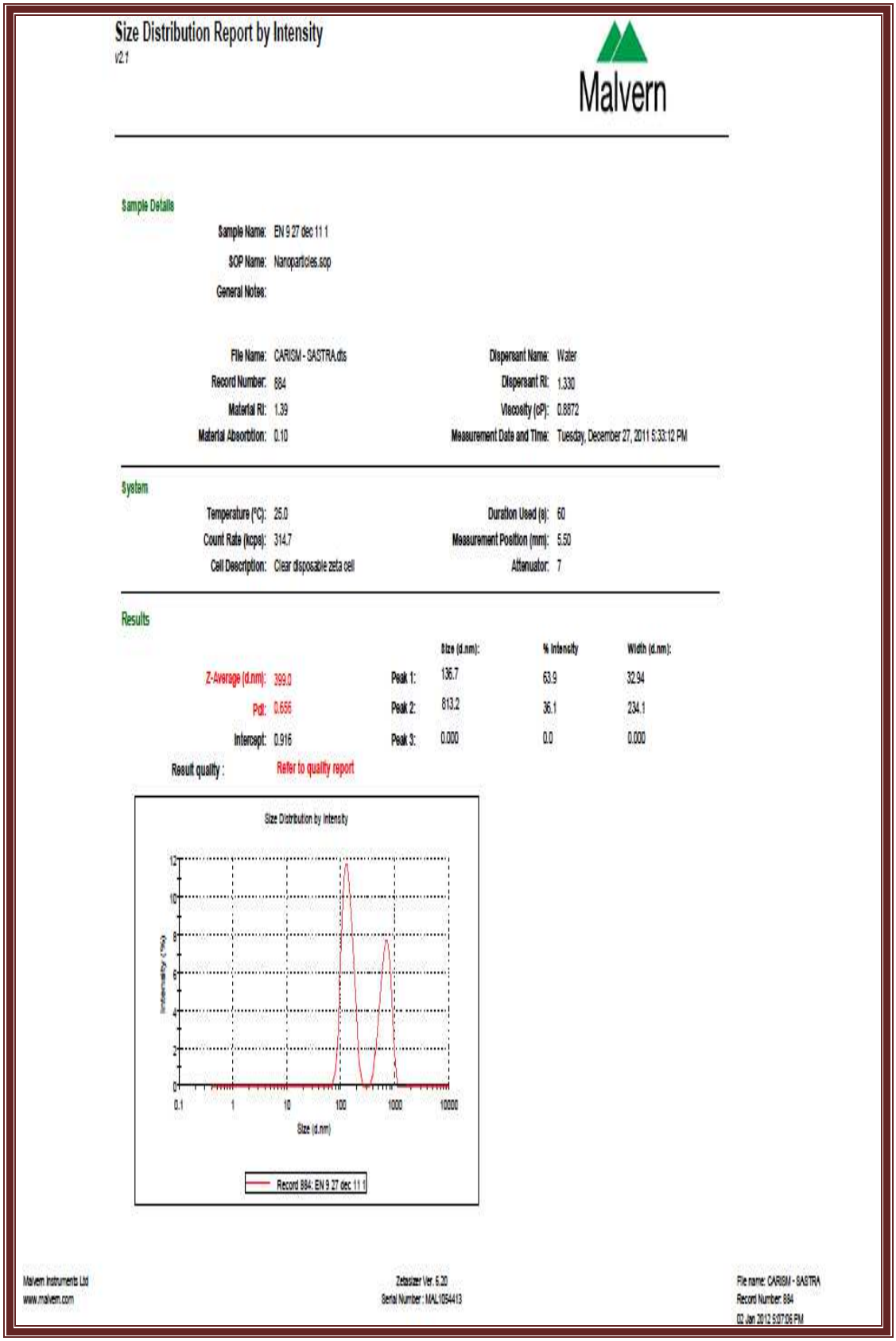
Serial Number : MAL1054413

File name: CARISM - SASTRA
Record Number: 882
02 Jan 2012 5:06:48 PM

**Fig.9.13:** Particle size distribution of formulation EN5



Size Distribution Report by Intensity
v2.1

Malvern

Sample Details

Sample Name: EN 9 27 dec 11 1
SOP Name: Nanoparticles.sop
General Notes:

File Name: CARISM - SASTRA.dts
Record Number: 884
Material RI: 1.39
Material Absorbtion: 0.10
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Tuesday, December 27, 2011 5:33:12 PM

System

Temperature (°C): 25.0
Count Rate (kcps): 314.7
Cell Description: Clear disposable zeta cell
Duration Used (s): 60
Measurement Position (mm): 5.50
Attenuator: 7

Results

| | | Size (d.nm): | % Intensity | Width (d.nm): |
|---|---|---|---|---|
| Z-Average (d.nm): 399.0 | Peak 1: | 136.7 | 63.9 | 32.94 |
| PdI: 0.656 | Peak 2: | 613.2 | 36.1 | 234.1 |
| Intercept: 0.916 | Peak 3: | 0.000 | 0.0 | 0.000 |

Result quality : Refer to quality report



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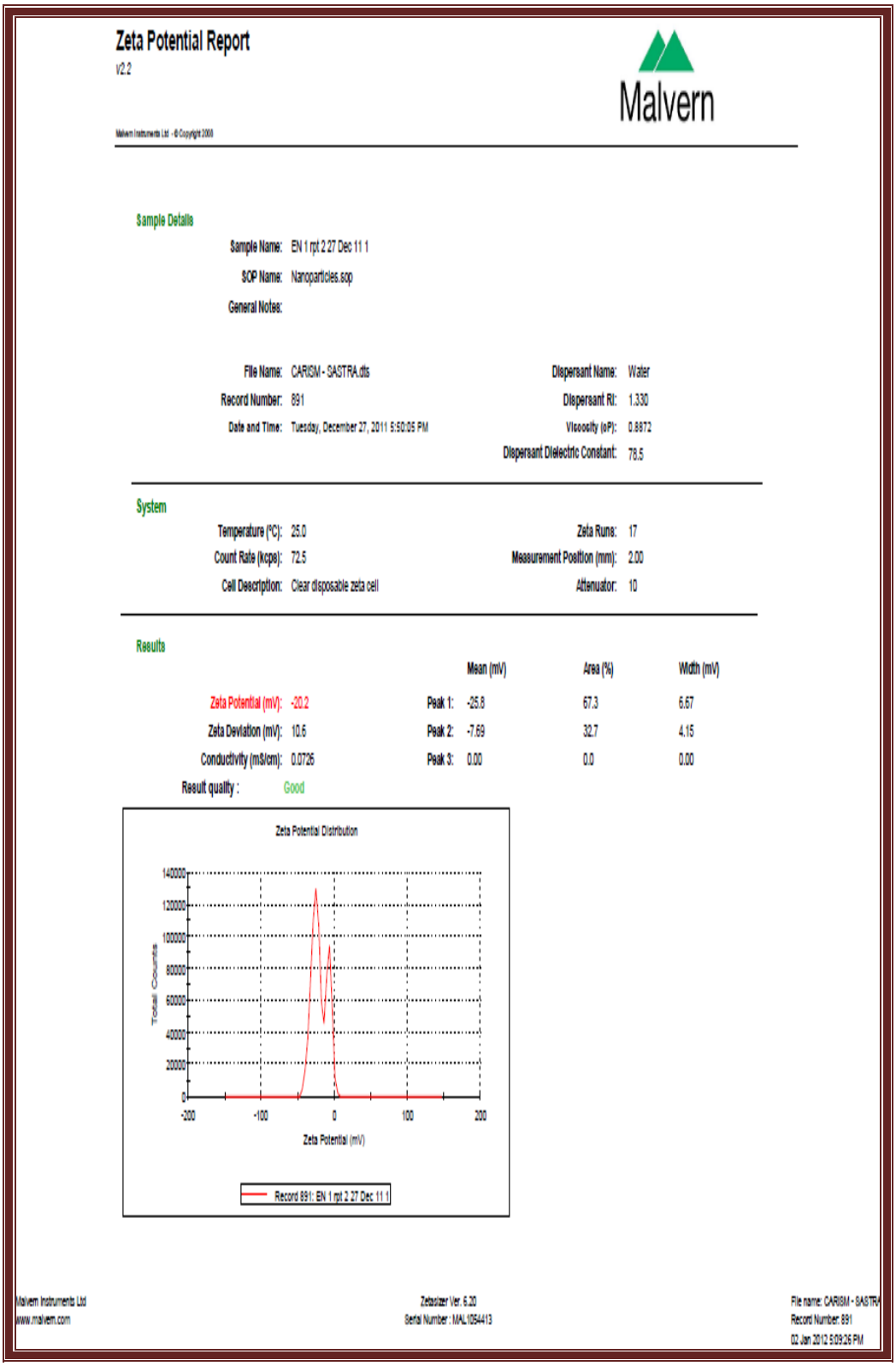
Serial Number : MAL1054413

File name: CARISM - SASTRA
Record Number: 884
02 Jan 2012 5:07:06 PM

**Fig.9.14:** Particle size distribution of formulation EN9

### 9.2.4. Determination of vesicle surface charge (Zeta potential):



Zeta Potential Report
v2.2

Malvern

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**Sample Details**

Sample Name: EN 1 rpt 2 27 Dec 11 1
SOP Name: Nanoparticles.sop
General Notes:

File Name: CARISM - SASTRA.dts
Record Number: 891
Date and Time: Tuesday, December 27, 2011 5:50:05 PM

Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

**System**

Temperature (°C): 25.0
Count Rate (kcps): 72.5
Cell Description: Clear disposable zeta cell

Zeta Runs: 17
Measurement Position (mm): 2.00
Attenuator: 10

**Results**

| | | Mean (mV) | Area (%) | Width (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): -20.2 | Peak 1: | -25.8 | 67.3 | 6.67 |
| Zeta Deviation (mV): 10.6 | Peak 2: | -7.69 | 32.7 | 4.15 |
| Conductivity (mS/cm): 0.0726 | Peak 3: | 0.00 | 0.0 | 0.00 |

Result quality : Good



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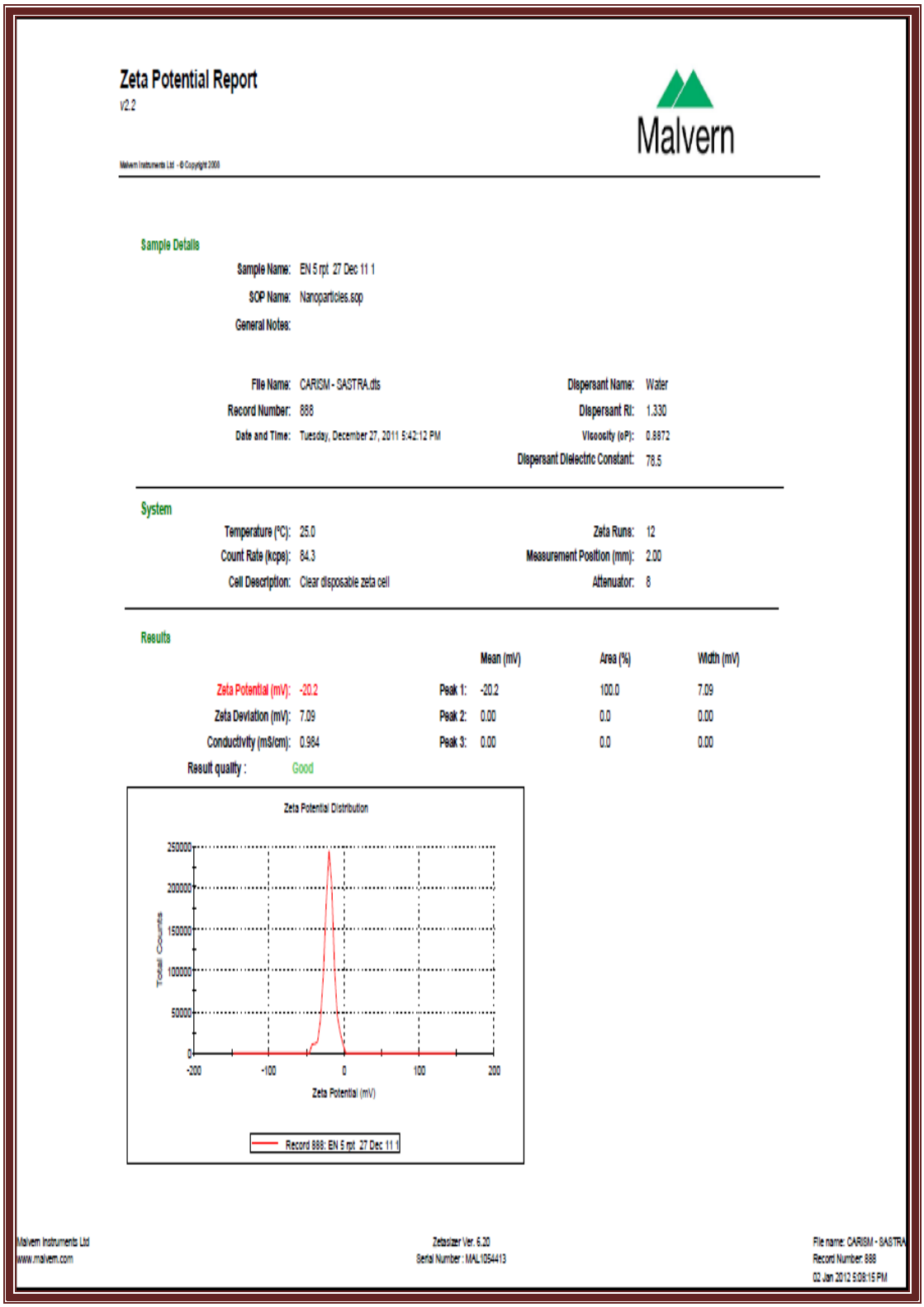
Serial Number : MAL1054413

File name: CARISM - SASTRA
Record Number: 891
02 Jan 2013 9:09:26 PM

**Fig.9.15:** Zeta potential report of Formulation EN1



Zeta Potential Report
v2.2

Malvern

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Sample Details

Sample Name: EN 5 rpt 27 Dec 11 1
SOP Name: Nanoparticles.sop
General Notes:

File Name: CARISM - SASTRA.dts
Record Number: 888
Date and Time: Tuesday, December 27, 2011 5:42:12 PM

Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Count Rate (kcps): 84.3
Cell Description: Clear disposable zeta cell

Zeta Runs: 12
Measurement Position (mm): 2.00
Attenuator: 8

Results

| | | Mean (mV) | Area (%) | Width (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): | -20.2 | Peak 1: -20.2 | 100.0 | 7.09 |
| Zeta Deviation (mV): | 7.09 | Peak 2: 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): | 0.984 | Peak 3: 0.00 | 0.0 | 0.00 |

Result quality : Good

Zeta Potential Distribution

Total Counts: 250000, 200000, 150000, 100000, 50000, 0
Zeta Potential (mV): -200, -100, 0, 100, 200

Record 888: EN 5 rpt 27 Dec 11 1

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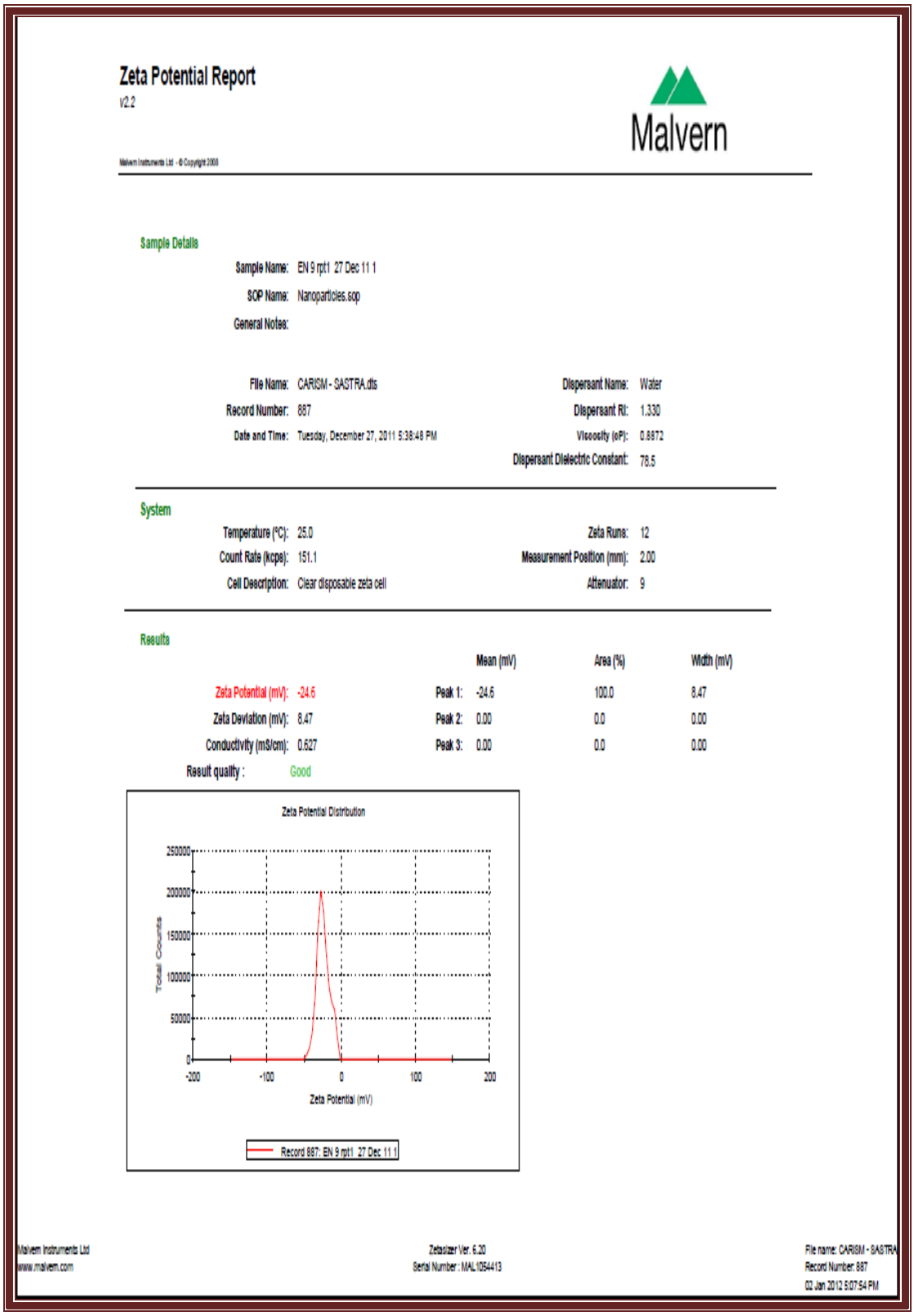
Zetasizer Ver. 6.20
Serial Number : MAL1054413

File name: CARISM - SASTRA
Record Number: 888
02 Jan 2012 5:08:15 PM

**Fig.9.16:** Zeta potential report of Formulation EN5



Zeta Potential Report
v2.2

Malvern

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Sample Details

Sample Name: EN 9 rpt1 27 Dec 11 1
SOP Name: Nanoparticles.sop
General Notes:

File Name: CARISM - SASTRA.dts
Record Number: 887
Date and Time: Tuesday, December 27, 2011 5:38:48 PM

Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Count Rate (kcps): 151.1
Cell Description: Clear disposable zeta cell

Zeta Runs: 12
Measurement Position (mm): 2.00
Attenuator: 9

Results

| | | Mean (mV) | Area (%) | Width (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): | -24.6 | Peak 1: -24.6 | 100.0 | 8.47 |
| Zeta Deviation (mV): | 8.47 | Peak 2: 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): | 0.627 | Peak 3: 0.00 | 0.0 | 0.00 |

Result quality : Good



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Zetasizer Ver. 6.20
Serial Number : MAL1054413

File name: CARISM - SASTRA
Record Number: 887
02 Jan 2012 5:07:54 PM

**Fig.9.17:** Zeta potential report of Formulation EN9

The stability study of the niosomes was evaluated by measuring the vesicle surface charge (zeta potential) of the niosomes by the zeta meter. The zeta potential reports were shown in Fig.9.15, 9.16 and 9.17.

Zeta potential of all formulated niosomes was in the range of −20.2 to – 24.5 mV which indicates moderate stability with no agglomeration.

### 9.2.5. Compatibility study:

#### 9.2.5.1. By Fourier transform infrared Spectroscopy (FTIR):

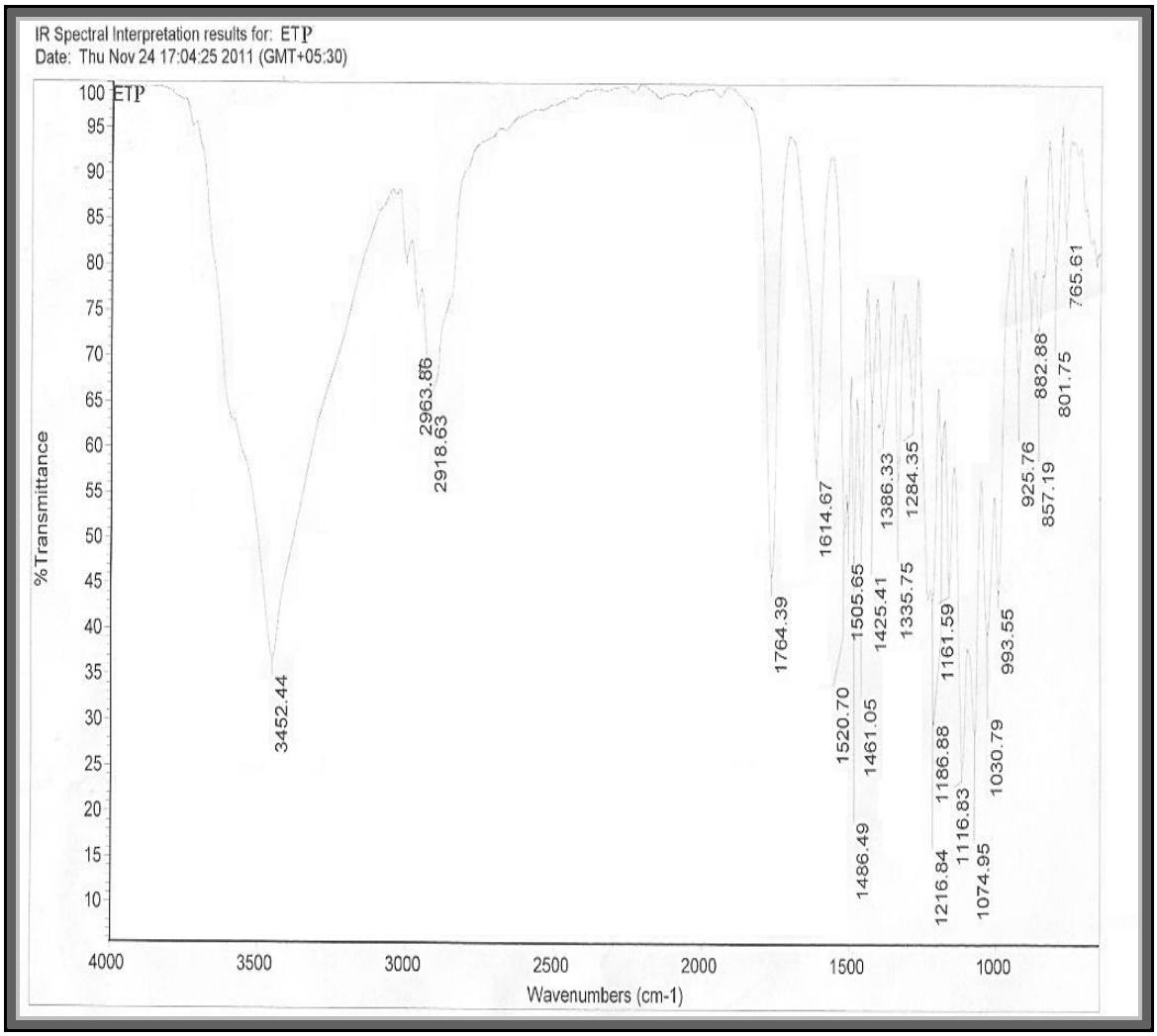


**Fig.9.18:** FTIR spectrum of pure Etoposide

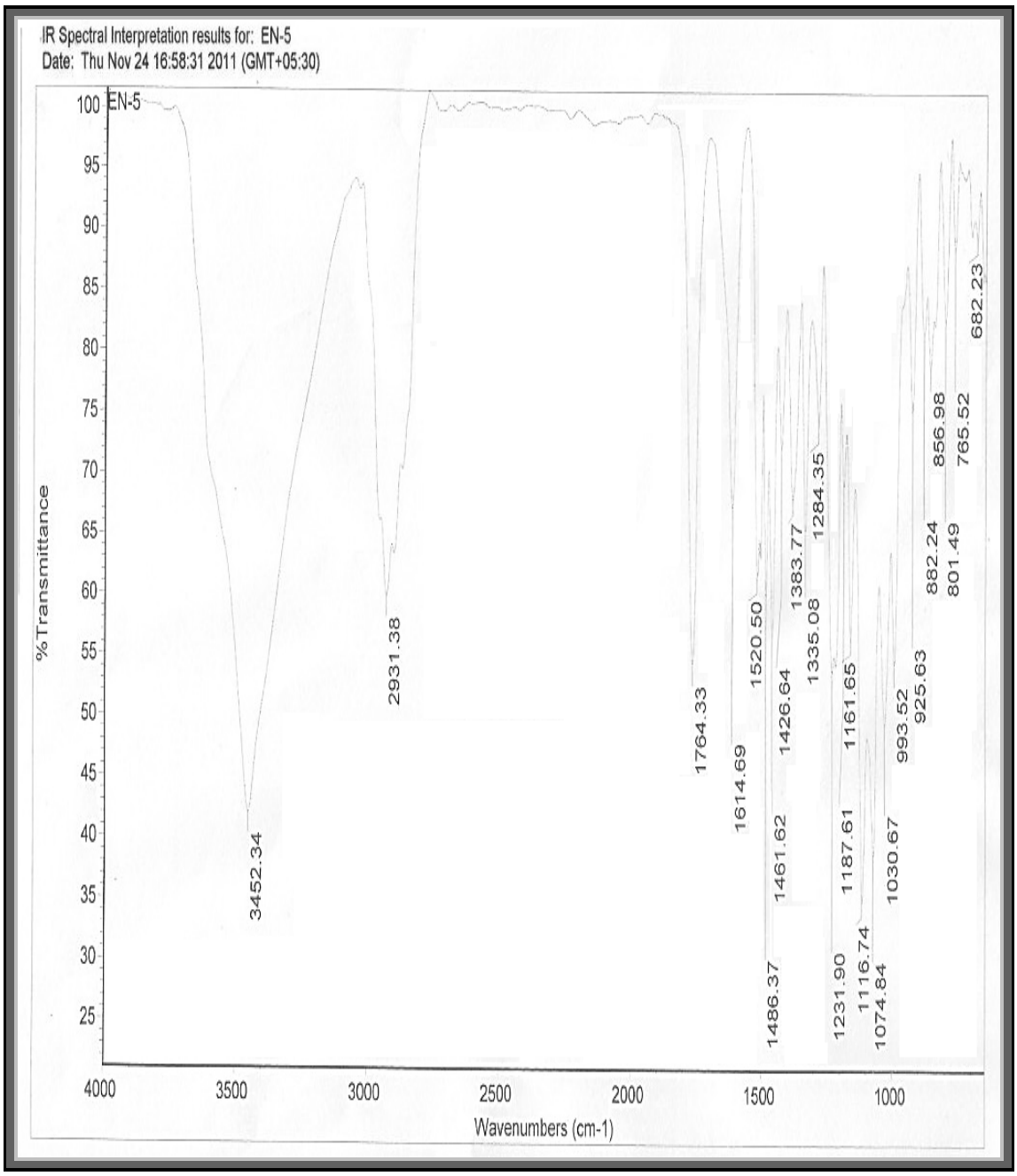


**Fig.9.19:** FTIR spectrum of Formulation EN5 containing Etoposide with Cholesterol and Surfactant

**Table 9.9:** Major peak observed in FTIR spectrum of pure Etoposide and Etoposide with cholesterol and surfactant used in best formulation.

| S.No | Wave No. ($cm^{-1}$) | Functional group | Peak observed (Yes/No) | |
|---|---|---|---|---|
| | | | Drug | Formulation EN5 |
| 1 | 3452.44 | O-H stretching | **Yes** | **Yes** |
| 2 | 2918.63 | $CH_3$ stretching | **Yes** | **Yes** |
| 3 | 1764.39 | C=O stretching | **Yes** | **Yes** |
| 4 | 1614.67 | C=C stretching | **Yes** | **Yes** |
| 5 | 1505.65 | C-C stretching | **Yes** | **Yes** |
| 6 | 1425.41 | COO stretching | **Yes** | **Yes** |
| 7 | 1335.75 | O-H stretching | **Yes** | **Yes** |
| 8 | 1284.35 | C-O stretching | **Yes** | **Yes** |
| 9 | 1116.83 | C-O-C stretching | **Yes** | **Yes** |
| 10 | 1074.95 | C-O bending | **Yes** | **Yes** |
| 11 | 993.55 | C-H bending | **Yes** | **Yes** |
| 12 | 882.88 | C-H bending | **Yes** | **yes** |
| 13 | 682 | C-C bending | **Yes** | **Yes** |

From the FTIR spectral analysis it was found that FTIR spectrum of Etoposide with cholesterol and surfactant showed all characteristic peaks in combination with no significant changes as shown in Fig.9.18 and 9.19. Therefore, it could indicate that there was no incompatibility between drug and excipients.

**9.2.5.2. By Differential scanning calorimetry (DSC) analysis:**

The compatibility and interactions between drug, cholesterol and surfactant were checked using DSC; results obtained were shown in Fig. 9.20 and 9.21.

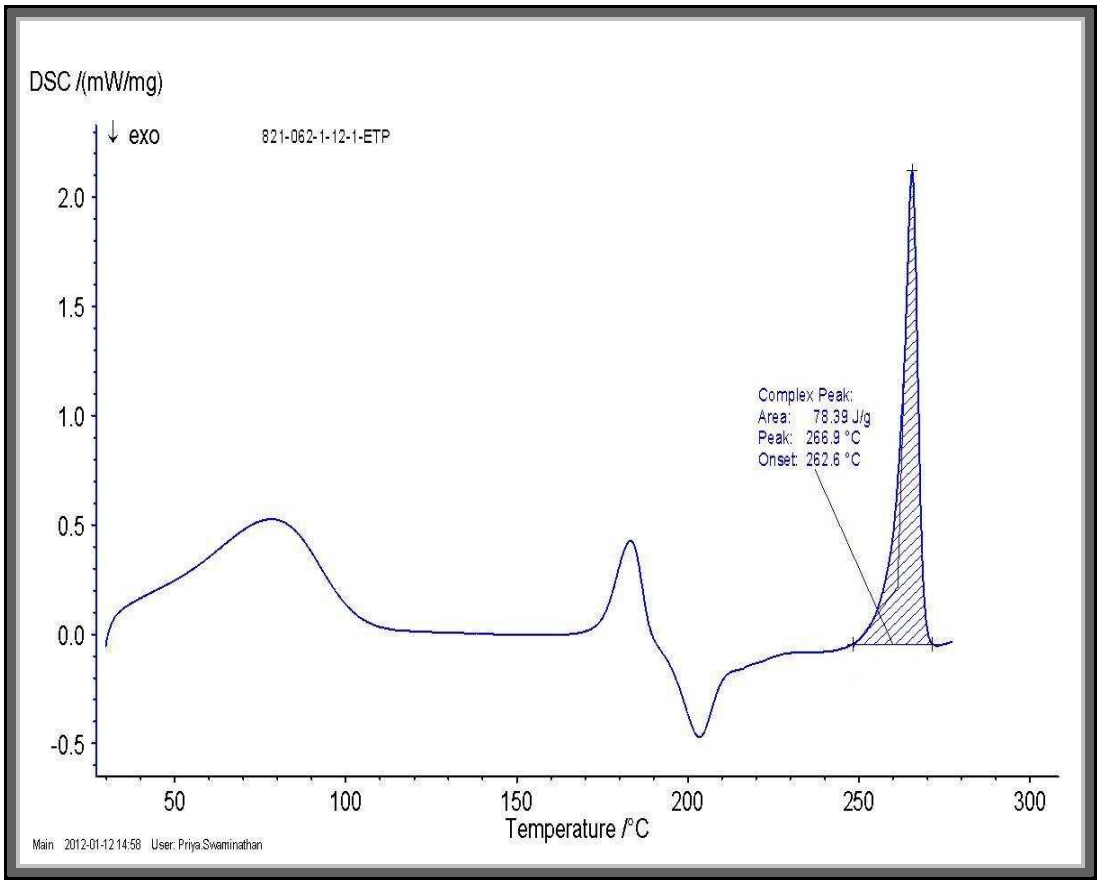


**Fig. 9.20:** DSC thermogram of Etoposide

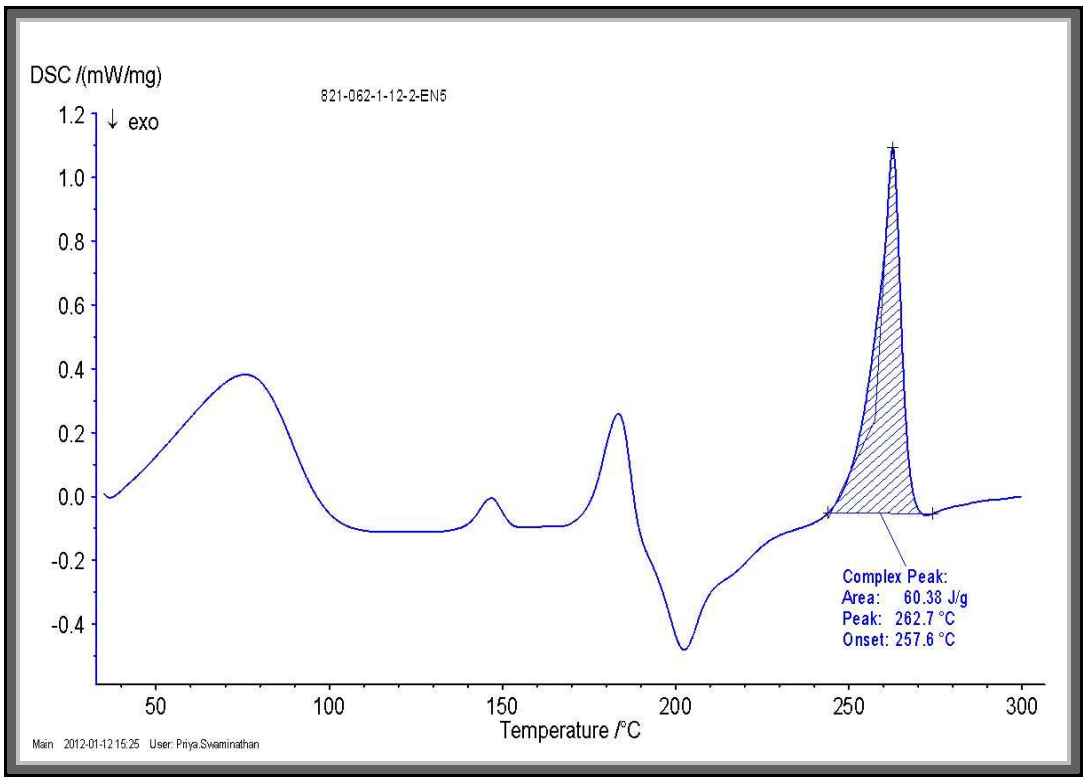


**Fig. 9.21:** DSC thermogram of Etoposide+ Cholesterol+Surfactant

**Table 9.10:** DSC thermogram parameters

| S. No. | DSC thermogram | Onset temperature (°C) | Peak temperature (°C) |
|---|---|---|---|
| 1 | Drug | 262.6 | 266.9 |
| 2 | Formulation EN5 | 257.6 | 262.7 |

According to Table 9.10, DSC thermogram showed that there was no major difference in onset temperature and peak temperature when compared with pure drug's thermogram. No interaction was found between drug and excipients. The DSC studies suggest that the formulation components polysorbate 80 and cholesterol and

the drug Etoposide do not interact to form any additional chemical entity but remain as a mixture. Therefore, it could indicate that there was no incompatibility between drug and excipients.

### 9.2.6. *In-vitro* drug release studies:

**Table 9.11:** *In-vitro* drug release profile of formulation EN1, EN2, EN3.

| S.No. | Time in hours | Medium | EN1 (%) | EN2 (%) | EN3 (%) |
|---|---|---|---|---|---|
| **1** | 1 | pH 7.4 PHOSPHATE BUFFER | 06.19 ± 0.48 | 03.95 ± 0.65 | 01.97 ± 0.33 |
| **2** | 2 | | 14.96 ± 0.65 | 09.90 ± 0.79 | 03.64 ± 0.39 |
| **3** | 3 | | 18.93 ± 0.94 | 14.75 ± 0.55 | 07.66 ± 0.31 |
| **4** | 4 | | 22.74 ± 1.02 | 19.66 ± 0.96 | 12.87 ± 0.65 |
| **5** | 5 | | 25.66 ± 0.63 | 23.47 ± 0.47 | 16.79 ± 0.39 |
| **6** | 6 | | 28.48 ± 1.10 | 25.81 ± 1.03 | 22.94 ± 0.39 |
| **7** | 7 | | 32.34 ± 0.71 | 30.88 ± 0.71 | 25.81 ± 1.41 |
| **8** | 8 | | 35.68 ± 1.10 | 33.75 ± 0.79 | 28.48 ± 0.87 |
| **9** | 9 | | 39.38 ± 1.20 | 36.82 ± 1.20 | 34.95 ± 0.80 |
| **10** | 10 | | 45.59 ± 0.39 | 44.96 ± 0.55 | 37.97 ± 0.79 |
| **11** | 24 | SALINE | 95.84 ± 0.86 | 89.74 ± 0.63 | 87.91 ± 0.89 |

All the values are expressed as mean± S.D., n=3.

**Table 9.12:** *In-vitro* drug release profile of formulation EN4, EN5, EN6.

| S.No. | Time in hours | Medium | EN4 (%) | EN5 (%) | EN6 (%) |
|---|---|---|---|---|---|
| **1** | 1 | pH 7.4 | 07.19 ± 0.87 | 05.88 ± 0.48 | 04.58 ± 0.24 |
| **2** | 2 | P | 09.43 ± 0.48 | 07.97 ± 0.31 | 05.20 ± 0.55 |
| **3** | 3 | H O | 12.87 ± 0.79 | 11.62 ± 0.39 | 07.97 ± 0.56 |
| **4** | 4 | S P | 16.53 ± 0.79 | 15.74 ± 0.48 | 14.75 ± 0.86 |
| **5** | 5 | H A | 25.87 ± 0.79 | 20.75 ± 0.33 | 17.67 ± 1.03 |
| **6** | 6 | T E | 28.79 ± 0.87 | 25.19 ± 0.41 | 23.52 ± 0.96 |
| **7** | 7 | B | 31.14 ± 0.31 | 29.26 ± 0.47 | 25.45 ± 0.80 |
| **8** | 8 | U F | 37.24 ± 0.72 | 32.75 ± 0.24 | 31.61 ± 0.78 |
| **9** | 9 | F E | 39.85 ± 0.48 | 37.82 ± 0.65 | 35.88 ± 0.80 |
| **10** | 10 | R | 46.69 ± 0.80 | 43.50 ± 0.56 | 41.78 ± 0.31 |
| **11** | 24 | SALINE | 94.38 ± 0.63 | 96.62 ± 0.39 | 91.35 ± 0.86 |

All the values are expressed as mean± S.D., n=3.

**Table 9.13:** *In-vitro* drug release profile of formulation EN7, EN8, EN9.

| S.No. | Time in hours | Medium | EN7 (%) | EN8 (%) | EN9 (%) |
|---|---|---|---|---|---|
| **1** | 1 | pH 7.4 | 09.33 ± 0.71 | 05.67 ± 0.59 | 04.16 ± 0.24 |
| **2** | 2 | PHOSPHATE BUFFER SALINE | 14.96 ± 0.94 | 08.23 ± 0.39 | 05.88 ± 0.55 |
| **3** | 3 | | 19.34 ± 0.71 | 11.99 ± 0.55 | 08.65 ± 0.96 |
| **4** | 4 | | 25.34 ± 0.87 | 16.53 ± 0.79 | 12.77 ± 0.48 |
| **5** | 5 | | 30.30 ± 1.01 | 19.87 ± 0.63 | 17.67 ± 0.87 |
| **6** | 6 | | 36.30 ± 0.78 | 24.30 ± 1.02 | 22.84 ± 0.87 |
| **7** | 7 | | 41.26 ± 0.55 | 29.21 ± 0.50 | 26.34 ± 1.02 |
| **8** | 8 | | 47.94 ± 1.26 | 33.48 ± 0.31 | 31.14 ± 0.87 |
| **9** | 9 | | 52.95 ± 0.86 | 37.87 ± 0.87 | 34.74 ± 1.18 |
| **10** | 10 | | 55.30 ± 0.55 | 43.97 ± 0.94 | 41.47 ± 0.31 |
| **11** | 24 | | 95.37 ± 0.63 | 93.86 ± 1.75 | 94.64 ± 0.79 |

All the values are expressed as mean± S.D., n=3.

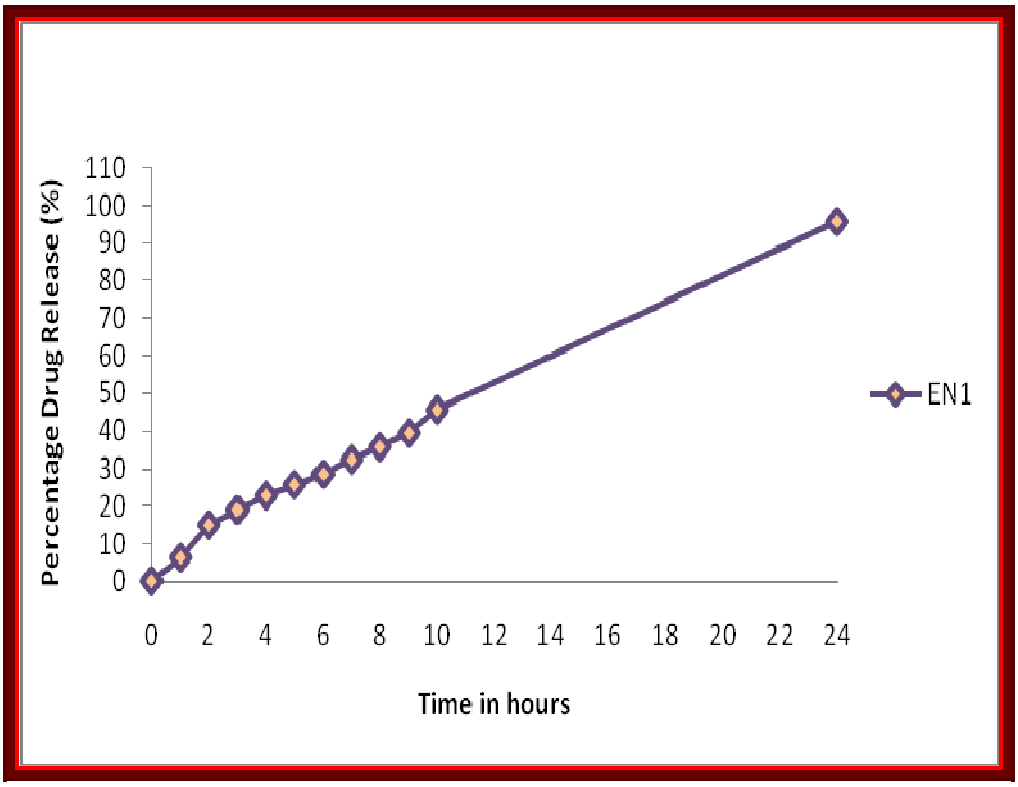


**Fig. 9.22:** *In-vitro* drug release profile of formulation EN1

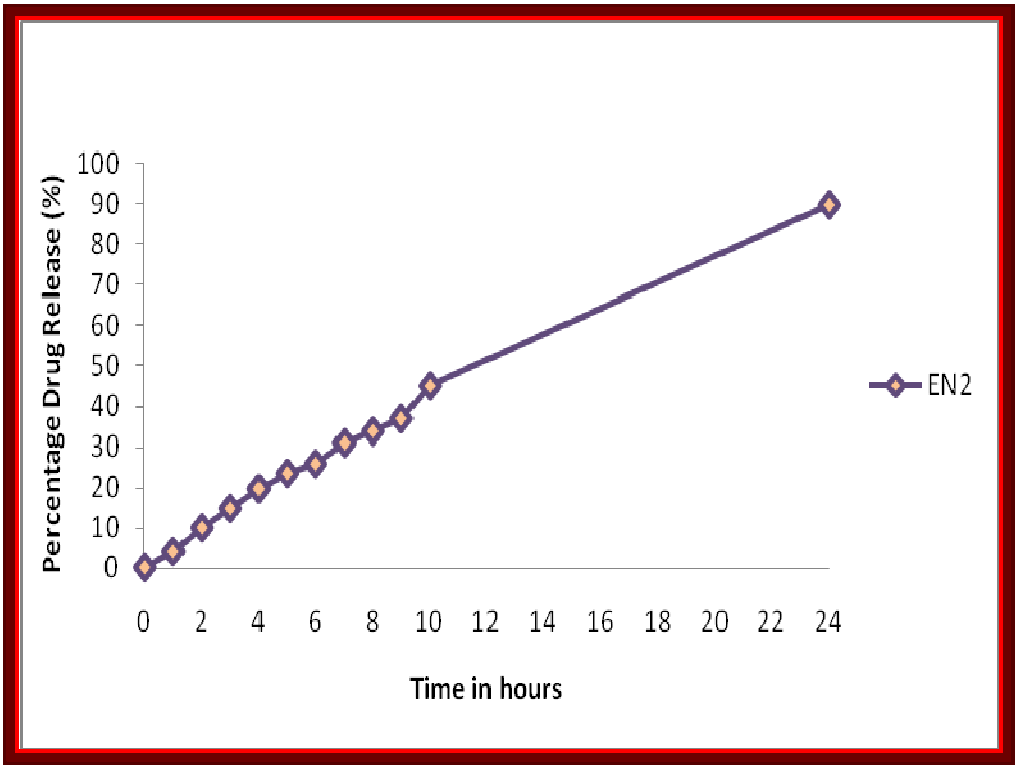


**Fig. 9.23:** *In-vitro* drug release profile of formulation EN2

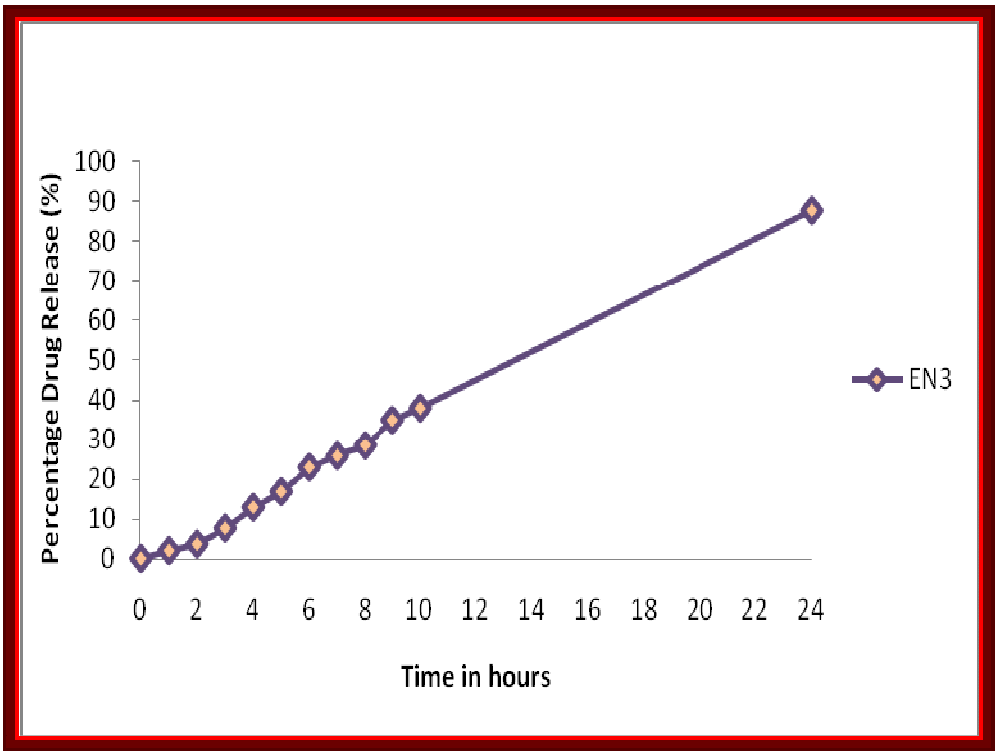


**Fig. 9.24:** *In-vitro* drug release profile of formulation EN3

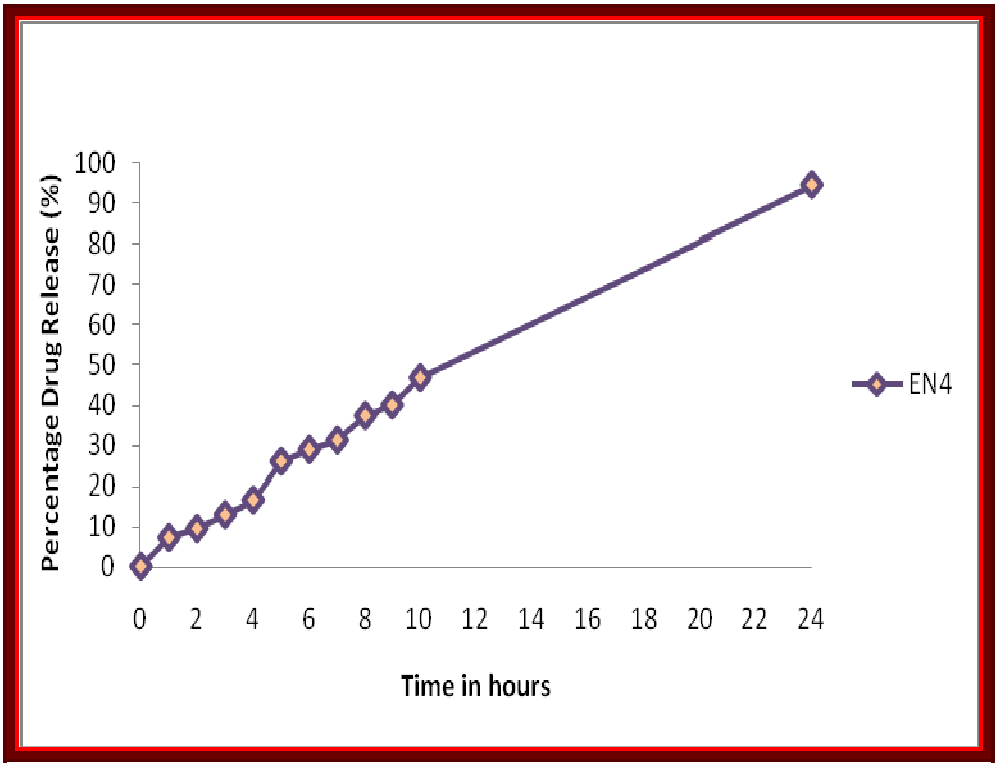


**Fig. 9.25:** *In-vitro* drug release profile of formulation EN4

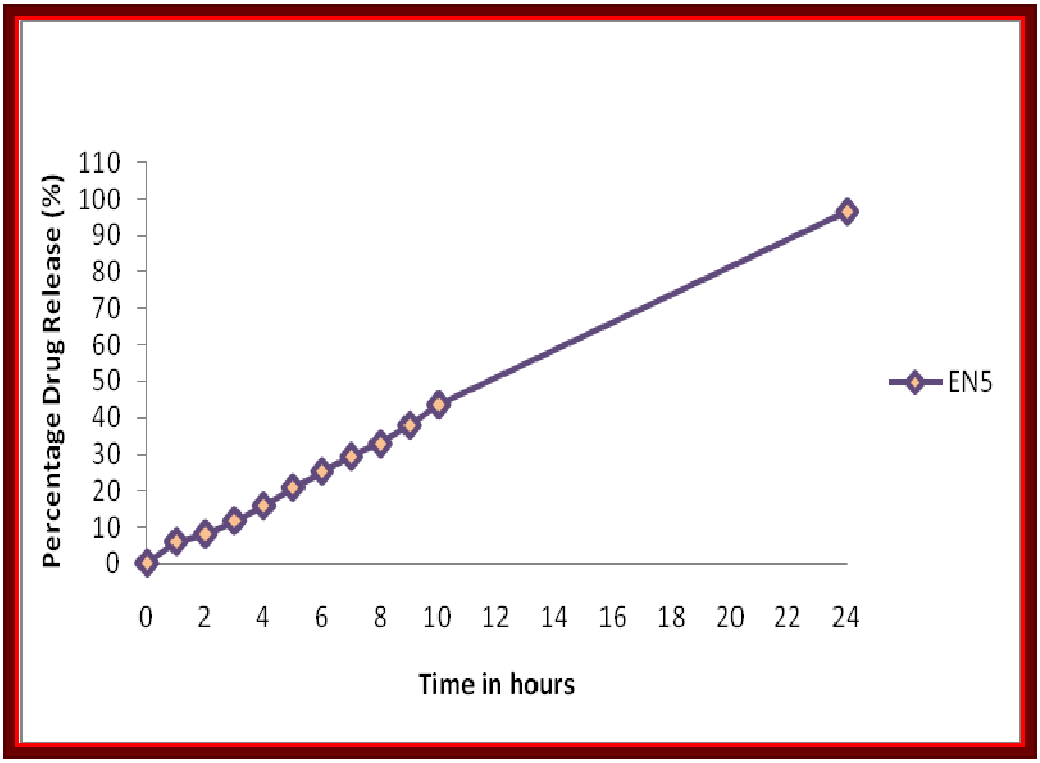


**Fig. 9.26:** *In-vitro* drug release profile of formulation EN5

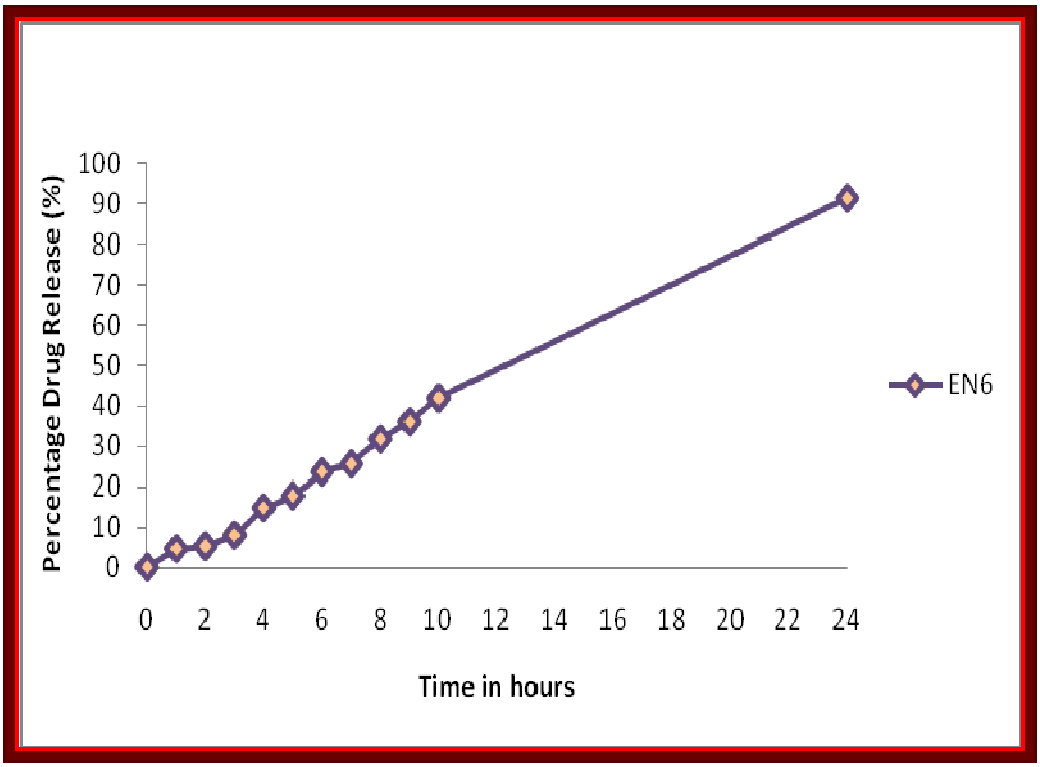


**Fig. 9.27:** *In-vitro* drug release profile of formulation EN6

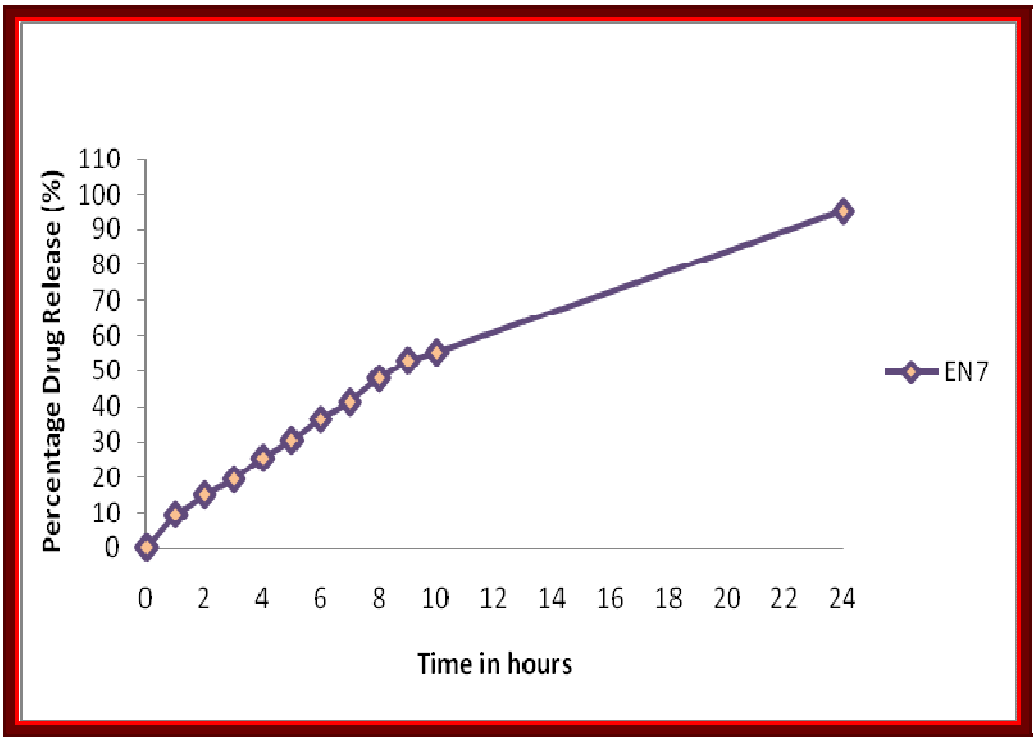


**Fig. 9.28:** *In-vitro* drug release profile of formulation EN7

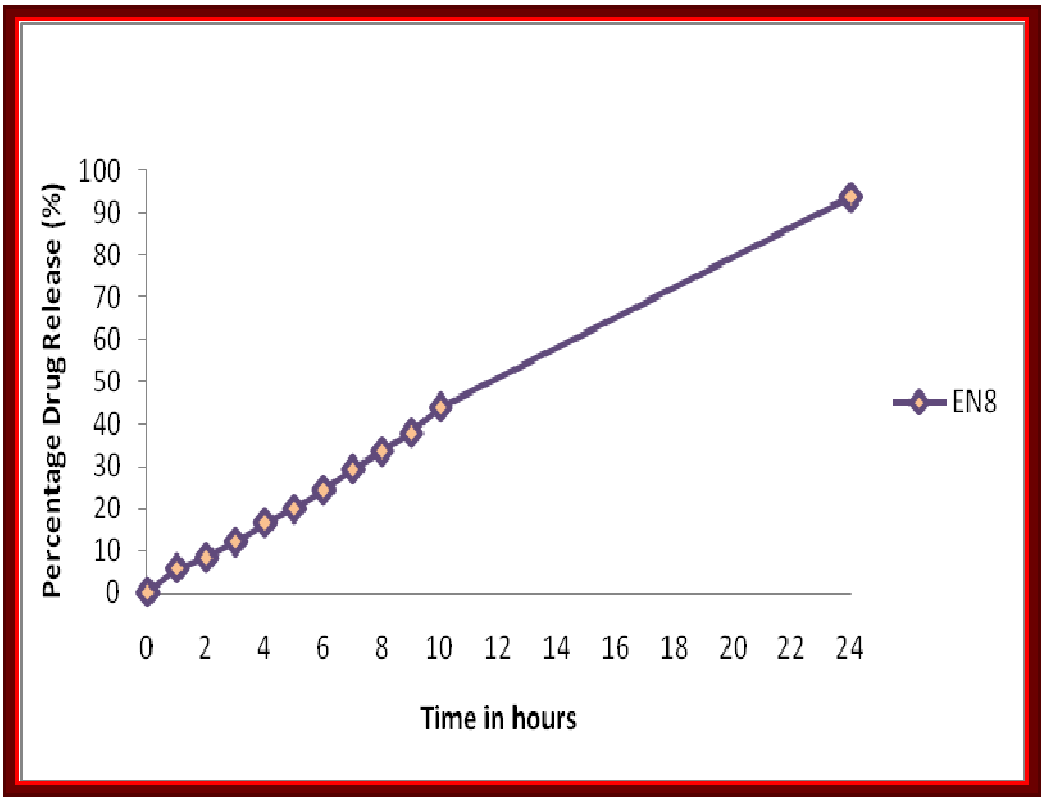


**Fig. 9.29:** *In-vitro* drug release profile of formulation EN8

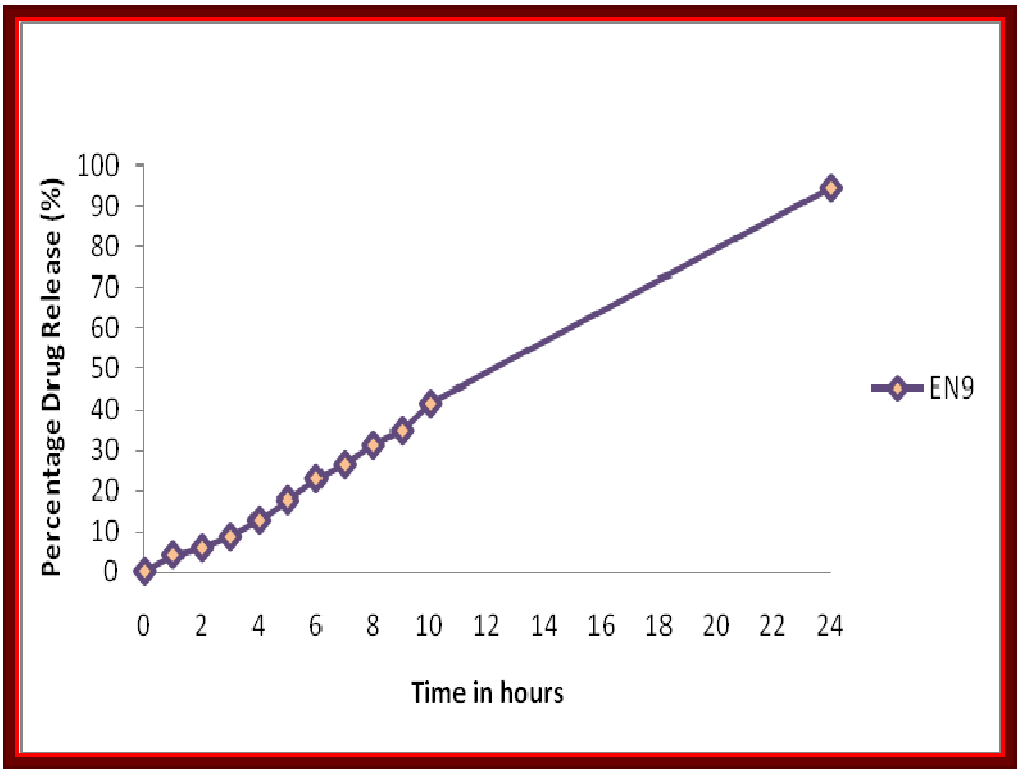


**Fig. 9.30:** *In-vitro* drug release profile of formulation EN9

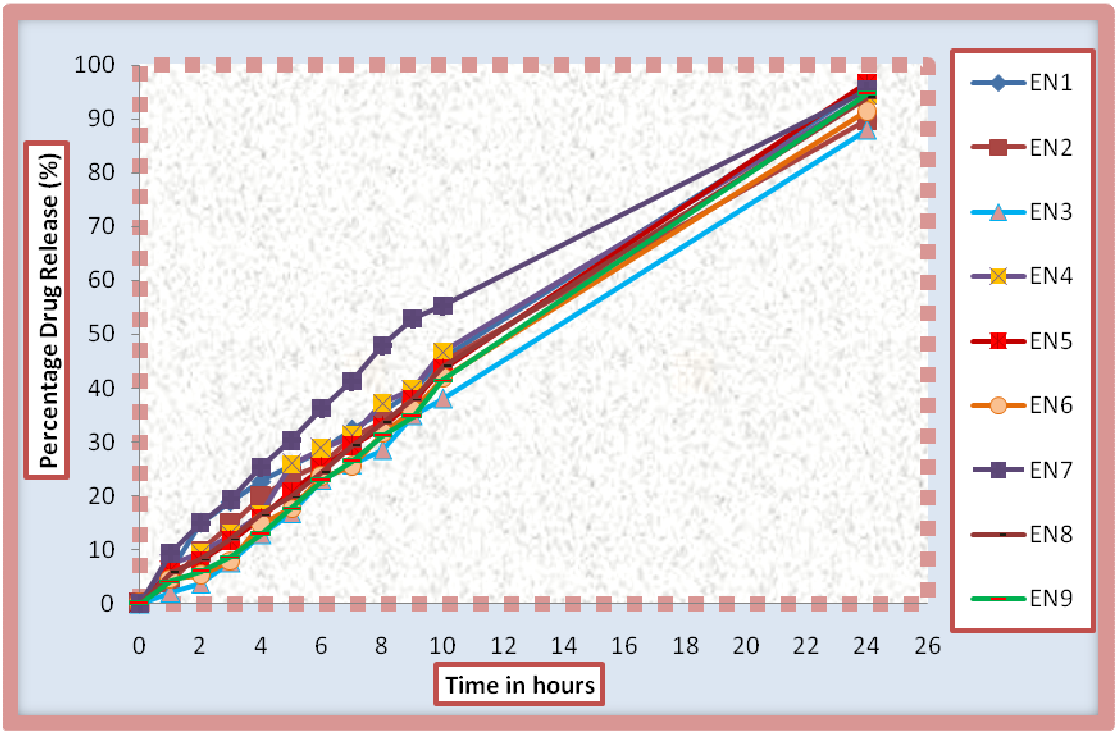


**Fig. 9.31:** Comparative *in-vitro* drug releases of all formulations

*In-vitro* dissolution studies revealed that the release of Etoposide from different formulations varies with the characteristics and composition of surfactant and cholesterol as shown in Figures 9.22 to 9.31.

The formulated niosomes showed a most favorable release within 24 hours. In the $24^{th}$ hour, the drug release was 95.84%, 89.74%, 87.91%, 94.38, 96.62, 91.35, 95.37, 93.86 and 94.64% for EN1, EN2, EN3, EN4, EN5, EN6, EN7, EN8 and EN9 respectively. This was followed by a steady drug release pattern.

From these above data, it showed formulation EN5 released drug mostly at the end of 24 hours. The release rate of Etoposide decreased with increasing concentration of Cholesterol. But increase in the rate of release was found with increasing amount of surfactant.

Among all the nine formulations, formulation EN5 containing surfactant and cholesterol in the molar ratio 1:1 exhibited the highest *in vitro* drug release of 96.62 at 24 hours, while the lowest drug release of 87.91 was recorded for formulation containing 0.5:1 molar ratio (EN3).

From the above evaluation parameters it was concluded that the **Formulation EN5** having a maximum percentage of drug release in a controlled manner, so the formulation EN5 was selected as the optimized formulation.

### 9.2.7. Kinetics of *In-vitro* drug release:

The kinetics of *in-vitro* drug release was determined by applying the drug release data to various kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas. The result obtained was shown in Table 9.14.

**Table 9.14:** Different kinetic models for Etoposide niosomes (EN1 to EN9)

| S. NO | F. Code | Zero order | First order | Higuchi | Korsemeyer-Peppas | | Best fit model |
|---|---|---|---|---|---|---|---|
| | | $R^2$ | $R^2$ | $R^2$ | $R^2$ | n | |
| 1 | EN1 | 0.991 | 0.839 | 0.918 | 0.983 | 0.801 | Zero |
| 2 | EN2 | 0.990 | 0.902 | 0.919 | 0.989 | 0.956 | Zero |
| 3 | EN3 | 0.995 | 0.870 | 0.875 | 0.983 | 1.271 | Zero |
| 4 | EN4 | 0.989 | 0.862 | 0.913 | 0.977 | 0.870 | Zero |
| 5 | EN5 | 0.998 | 0.800 | 0.884 | 0.985 | 0.932 | Zero |
| 6 | EN6 | 0.994 | 0.853 | 0.876 | 0.965 | 1.050 | Zero |
| 7 | EN7 | 0.948 | 0.924 | 0.964 | 0.992 | 0.769 | Peppas |
| 8 | EN8 | 0.996 | 0.844 | 0.891 | 0.989 | 0.928 | Zero |
| 9 | EN9 | 0.997 | 0.810 | 0.864 | 0.982 | 1.058 | Zero |

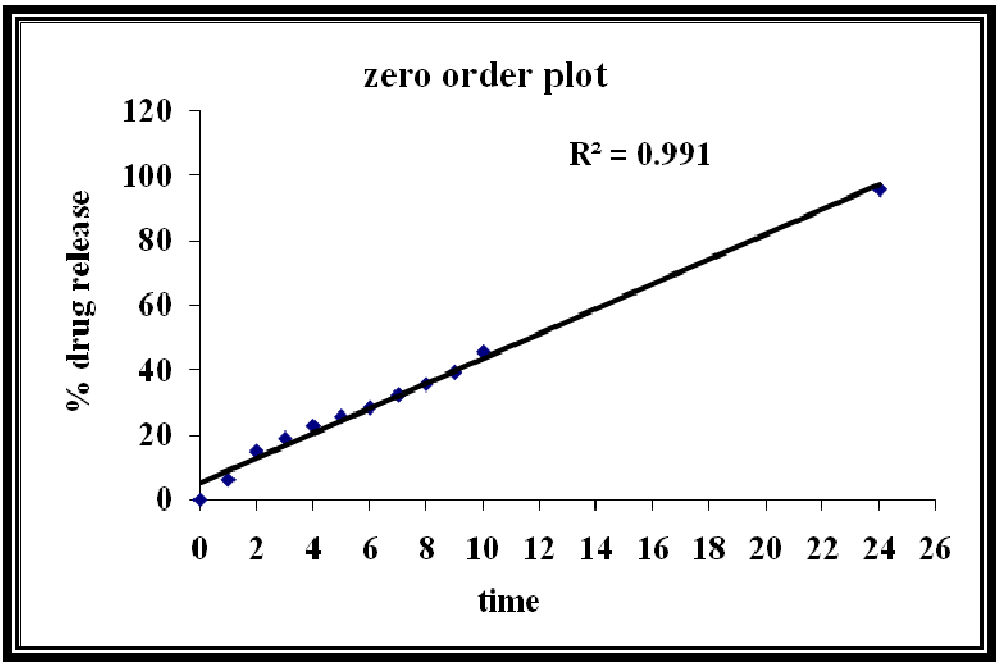


**Fig. 9.32:** Best fit kinetic release of formulation EN1

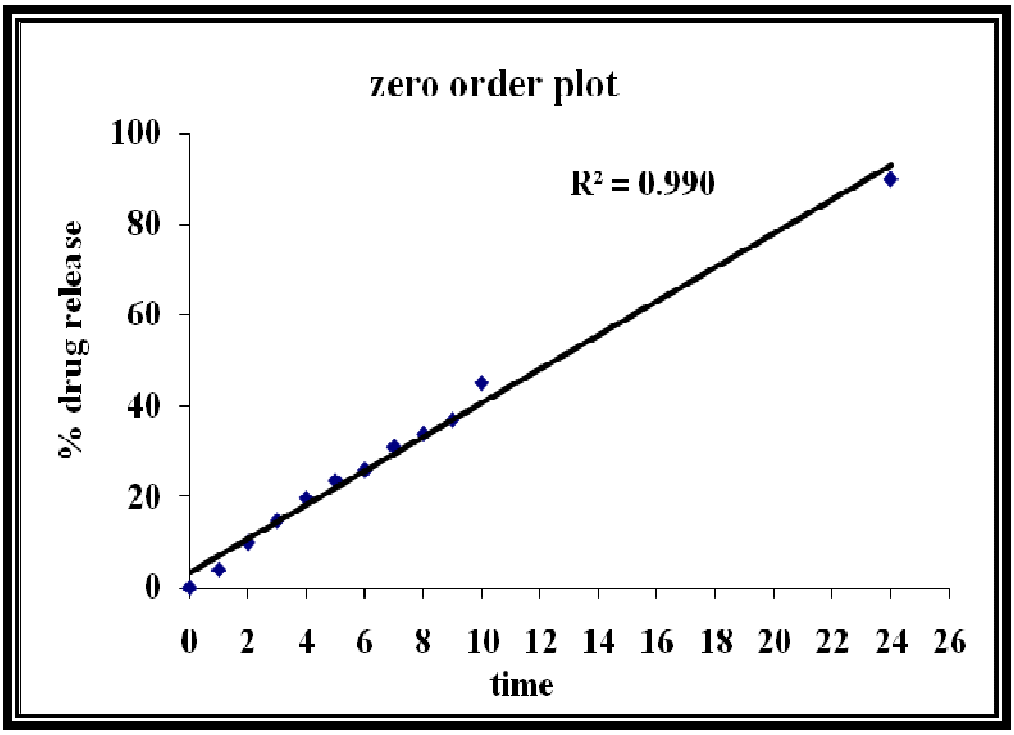


**Fig. 9.33:** Best fit kinetic release of formulation EN2

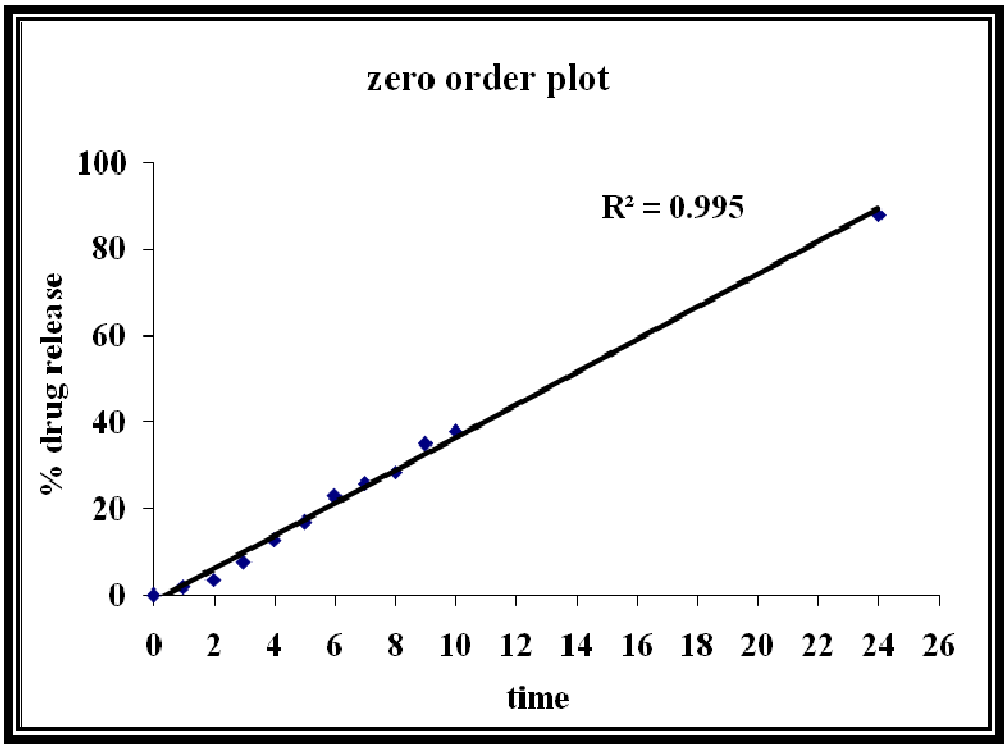


**Fig. 9.34:** Best fit kinetic release of formulation EN3

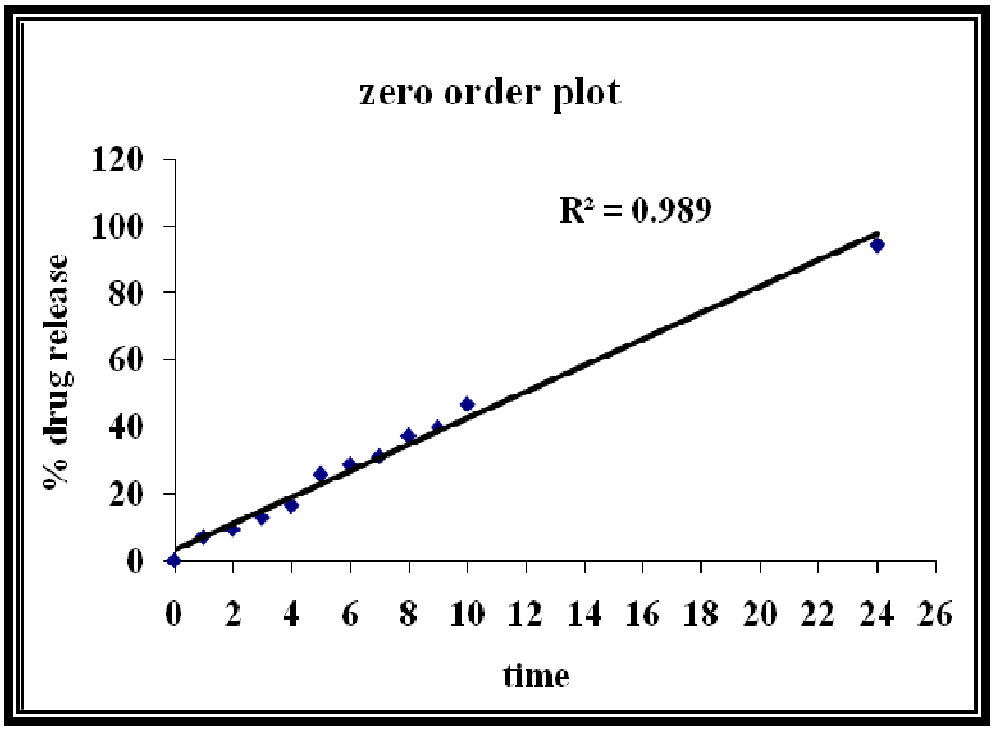


**Fig. 9.35:** Best fit kinetic release of formulation EN4

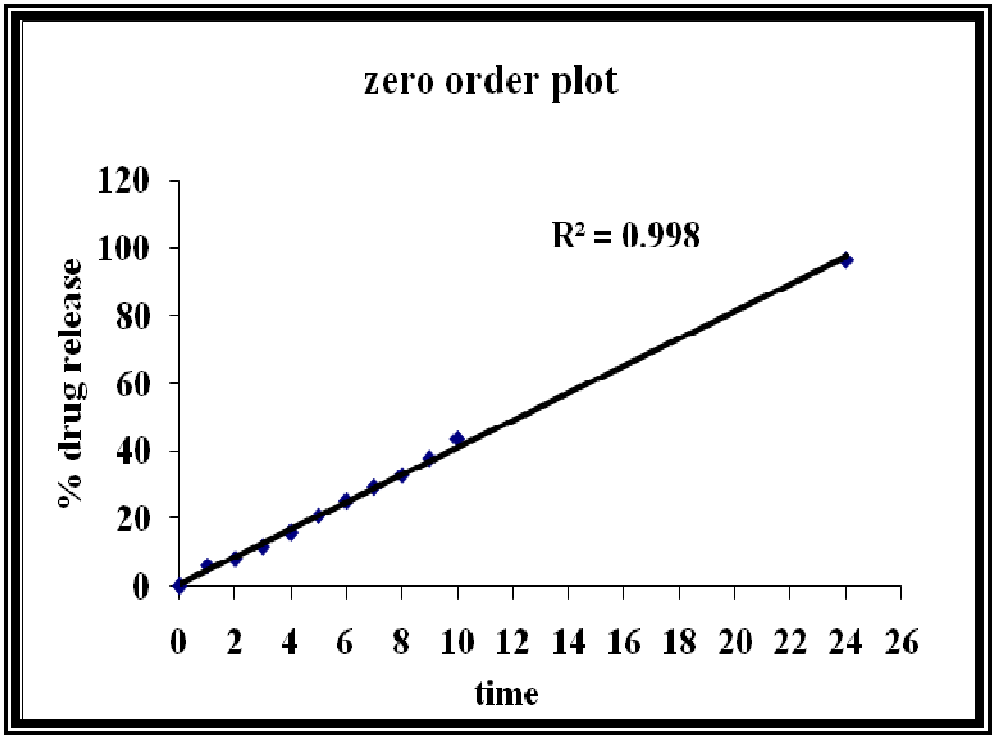


**Fig. 9.36:** Best fit kinetic release of formulation EN5

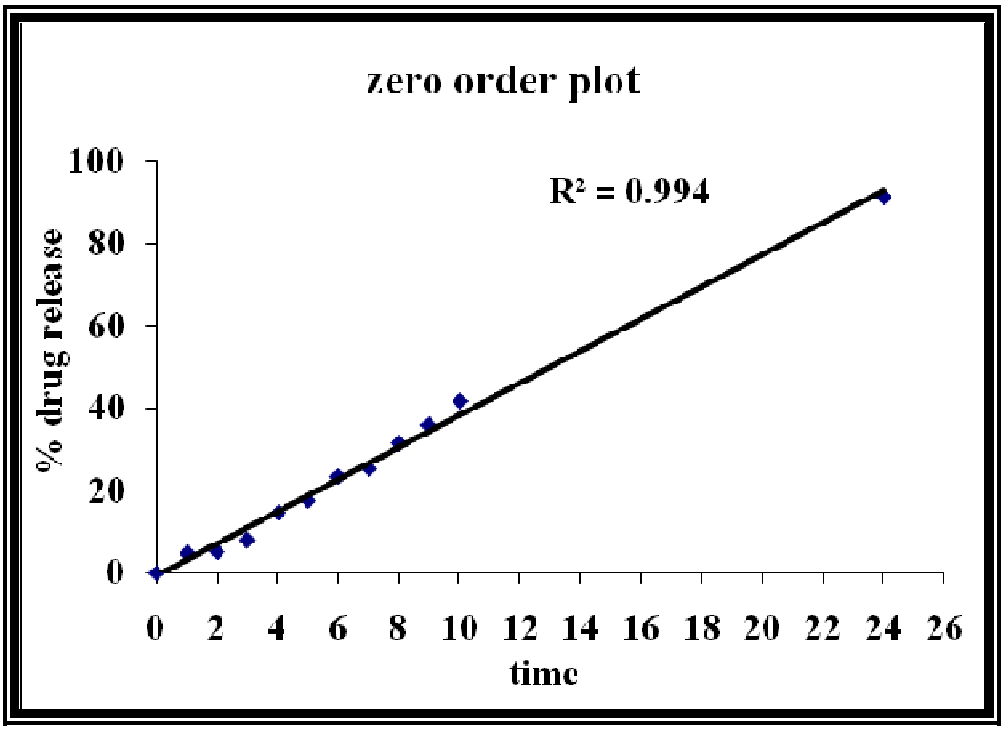


**Fig. 9.37:** Best fit kinetic release of formulation EN6

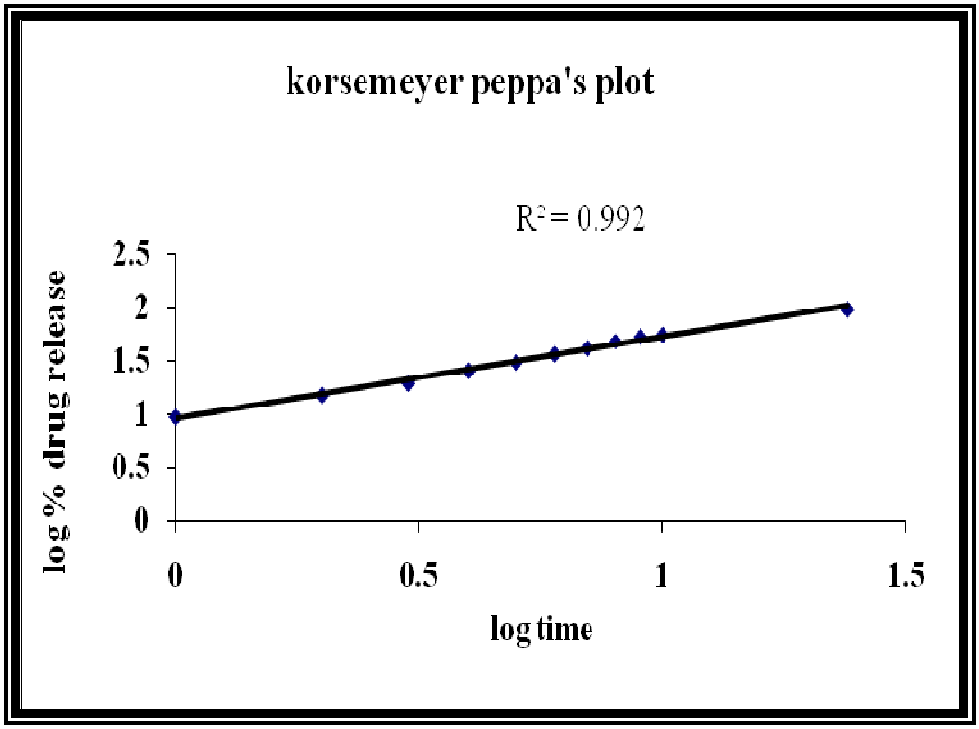


**Fig. 9.38:** Best fit kinetic release of formulation EN7

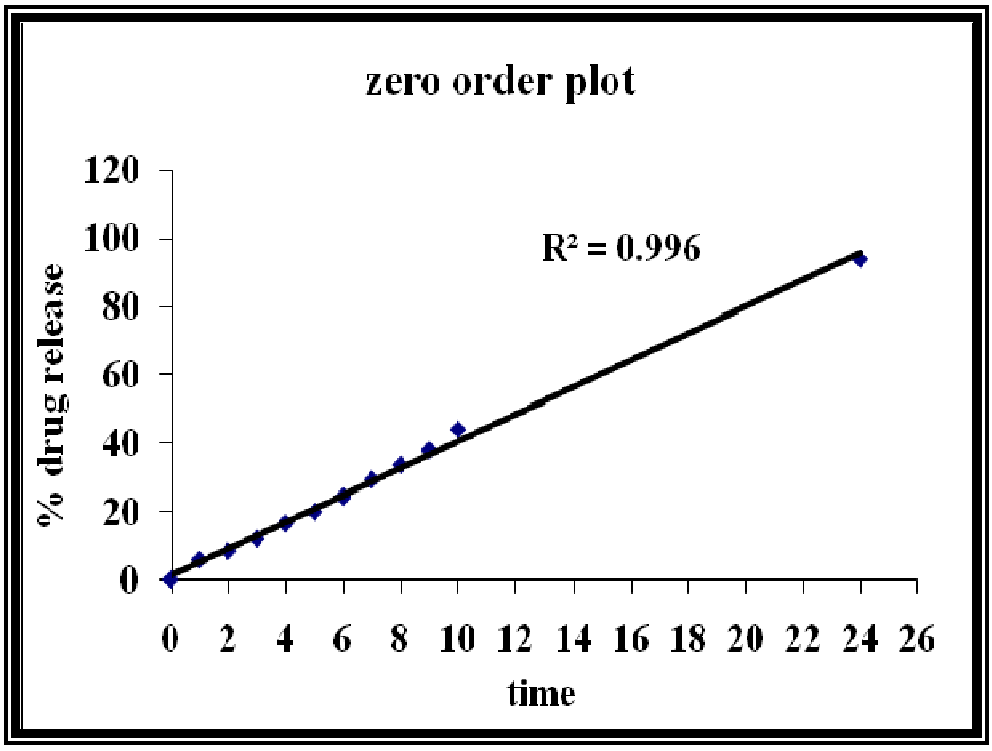


**Fig. 9.39:** Best fit kinetic release of formulation EN8

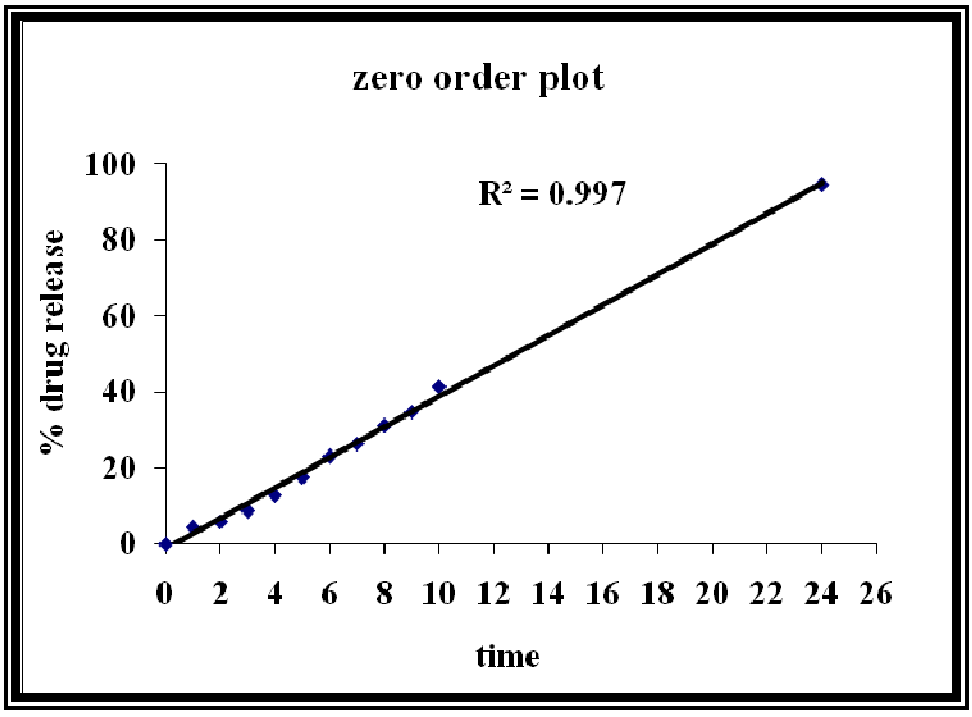


**Fig. 9.40:** Best fit kinetic release of formulation EN9

### 9.2.8. Test for sterility:

After *in-vitro* drug release studies, best formulation was selected. The selected formulation was sterilized by membrane filteration. Then sterility testing was performed and the results were reported in Table 9.15.

Growth of micro organisms was noticed during the seven days of incubation period in sterilized niosomal formulation and there was no growth.

Thus, sterilized niosomal formulation passes the test for sterility and there was no growth which indicates niosomes can be administered by parenteral route.

**Table 9.15:** Sterility testing of selected formulation (EN-5)

| S.No. | Type | Growth of micro organism |
|---|---|---|
| 1 | Negative control | Presence |
| 2 | Sterilized formulation | Absent |

### 9.2.9. *In- vivo* drug targeting studies:

The *in- vivo* drug targeting was estimated by performing *In- vivo* tissue distribution study for an optimized formulation EN 5. The percentage drug recovered in the tissues of various organs for Etoposide niosomes were compared with that of free Etoposide. The comparison between the amount of drug targeted from niosomes (formulation with highest drug content) and pure drug Etoposide in various organs is represented.

The results obtained were recorded in Table 9.16 and 9.17 and shown in Fig. 9.41, 9.42 and 9.43 respectively.

**Table 9.16:** *In-vivo* tissue distribution studies of Etoposide niosomes EN5

| S. No | Organs | Percentage drug recovered in tissues* (%) |
|---|---|---|
| 1. | Liver | 41.56 ±0.87 |
| 2. | Spleen | 25.38 ±0.62 |
| 3. | Lung | 23.74 ±0.54 |
| 4. | Kidney | 21.24 ±0.40 |
| 5. | Brain | 4.76 ±0.76 |

All the values were expressed as mean S.D., *n=5

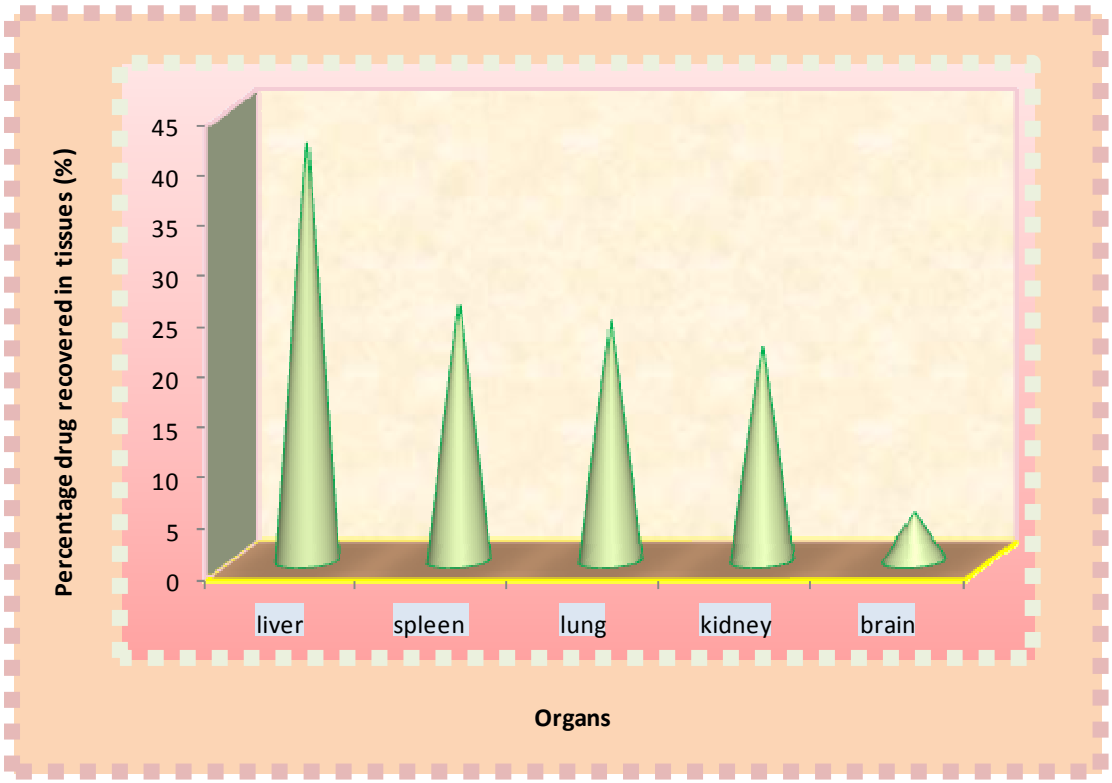


**Fig. 9.41:** *In-vivo* tissue distribution studies of Etoposide niosomes EN5

**Table 9.17:** *In- vivo* tissue distribution studies of pure Etoposide.

| S. No | Organs | Percentage drug recovered in tissues* (%) |
|---|---|---|
| 1. | liver | 28.46 ±0.62 |
| 2. | Spleen | 16.32 ±0.58 |
| 3. | Lung | 13.63 ±0.73 |
| 4. | Kidney | 11.89 ±0.71 |
| 5. | Brain | 3.63 ±0.51 |

All the values were expressed as mean S.D., *n=5

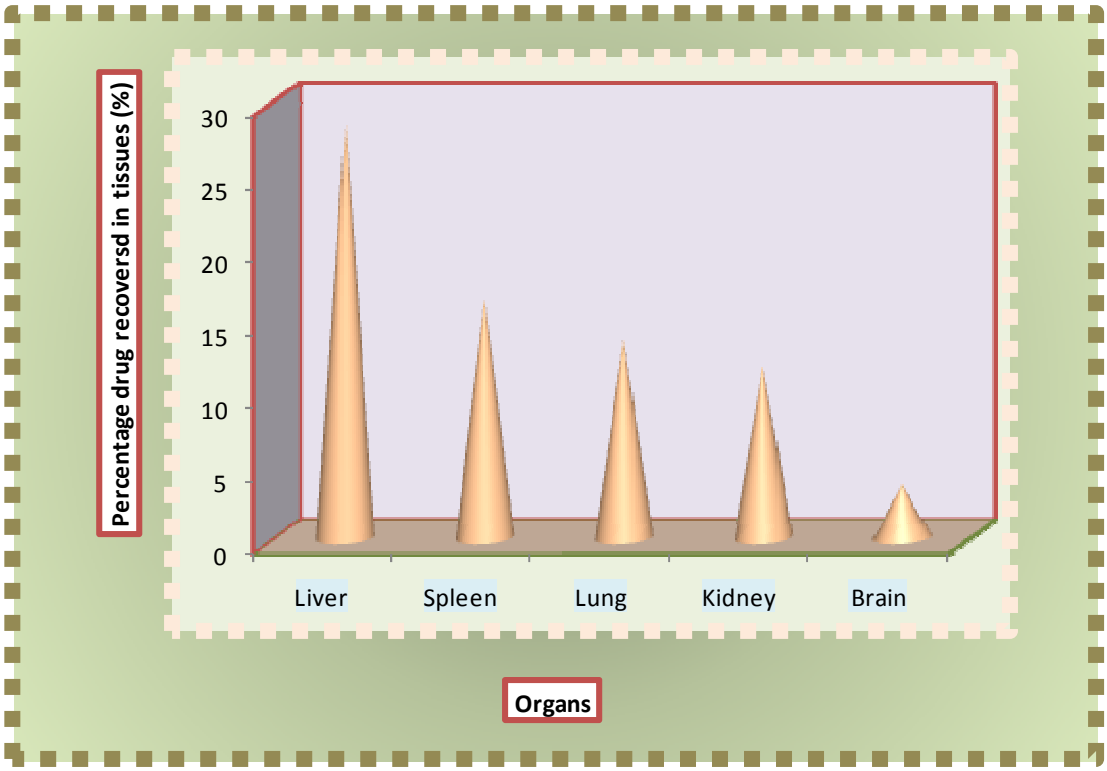


**Fig. 9.42:** *In-vivo* tissue distribution studies of pure Etoposide

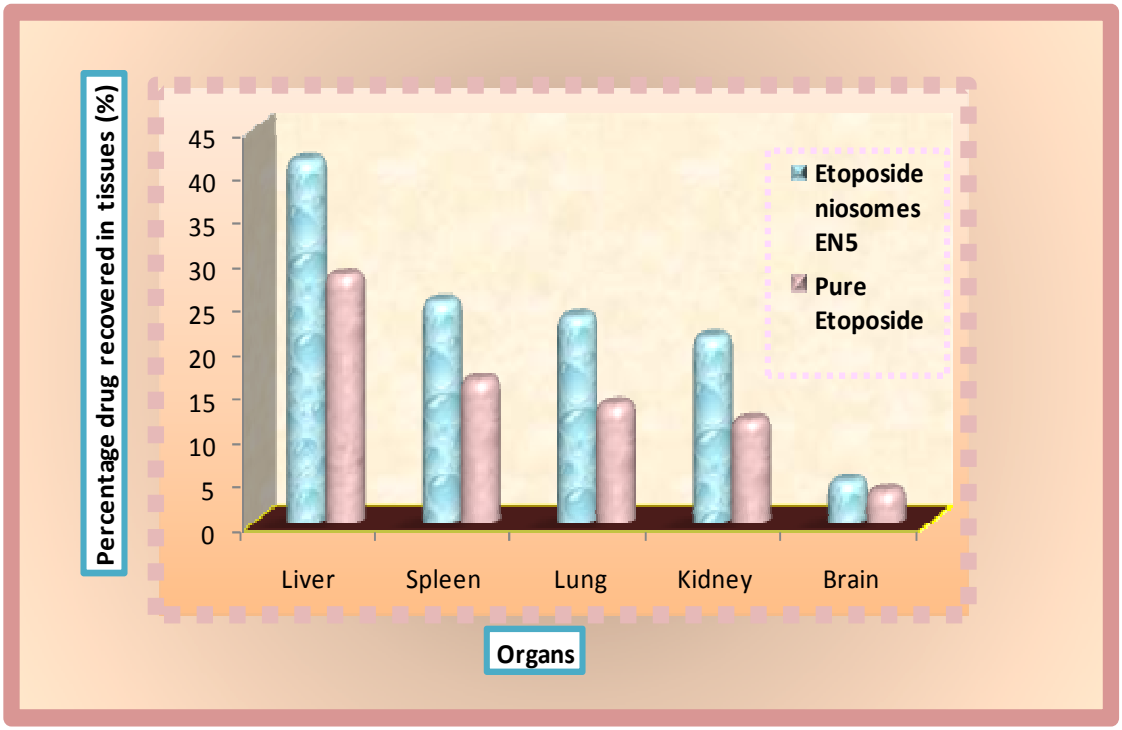


**Fig. 9.43:** Comparison of *In-vivo* tissue distribution studies of Etoposide niosomes EN5 with pure Etoposide

The average targeting efficiency of drug loaded niosomes was found to be 41.56% of the injected as dose in liver, 25.38% in spleen, 23.74% in lungs, 21.24% in kidney, 4.76% in brain as compared to the concentration of pure drug was 28.46% in liver, 16.32% in spleen, 13.63% in lungs, 11.89% in kidney, 3.63% in brain. The drug loaded niosomes showed preferential drug targeting to liver followed by spleen, lungs, kidney and brain.

These results revealed that, the drug loaded niosomes showed preferential drug targeting to liver followed by spleen, lungs, kidney and brain. It was also revealed that, as compared to pure drug, higher concentration of drug was targeted to the organs like liver and lungs after administering the dose in the form of niosomes. This may lead to attributed to high macrophage load in these organs and large size of liver as compared to spleen and lungs.

### 9.2.10. Stability studies:

After exposure to refrigerator temperature, room temperature and accelerated stability conditions (4ºC ±1ºC , 25ºC± 2ºC at 60% RH±5%RH and 40ºC ±2ºC at 75% RH ±5% RH) the formulation was analyzed for various evaluation parameters; results are shown in Table 9.18-9.23.

**Table 9.18:** Stability studies of optimized formulation EN5 at refrigerator temperature

| Characteristic | Initials | 1st Month | 2nd Month | 3th Month |
|---|---|---|---|---|
| **Drug retained (%)** | 92.10 | 91.85 | 91.46 | 91.33 |
| **In vitro drug release at 24 hour* (%)** | 96.62±0.39 | 96.57±0.27 | 95.79±0.72 | 94.59±0.79 |
| **Test for sterility** | Sterile | Sterile | Sterile | Sterile |

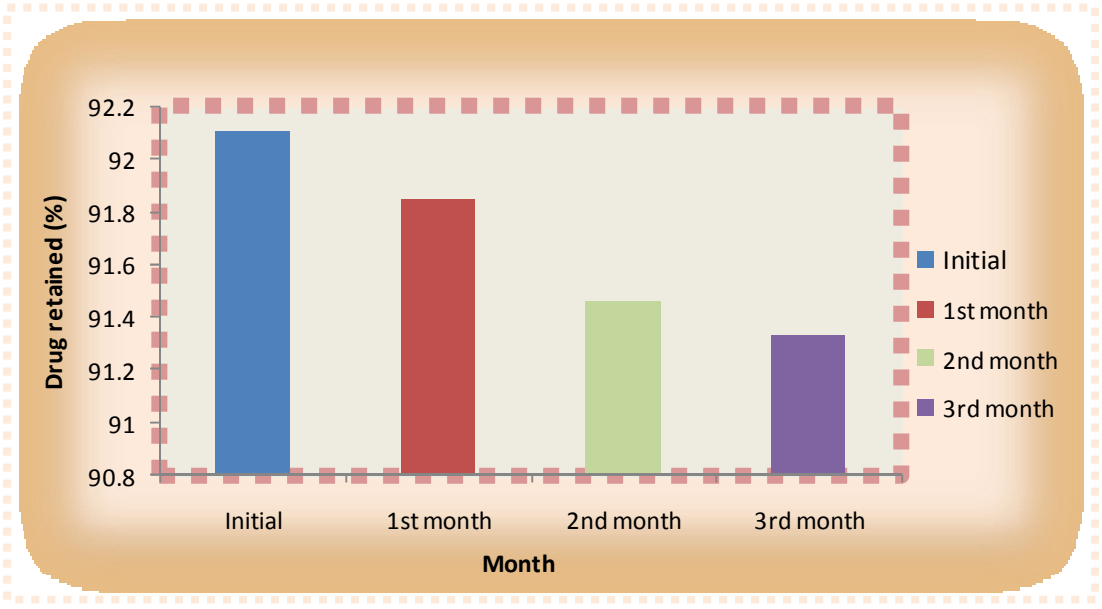


**Fig. 9.44:** Comparison of drug retained (%) before and after stability studies at refrigerator temperature for optimized formulation EN5

**Table 9.19:** Percentage *in-vitro* drug release of selected formulation EN5 after stability studies at refrigerator temperature

| S. No | Time hours | Refrigerator temperature | | | |
|---|---|---|---|---|---|
| | | Initial* | 1st month* | 2nd month* | 3rd month* |
| 1 | 1 | 5.88±0.48 | 5.933±0.54 | 6.037±0.48 | 4.107±0.39 |
| 2 | 2 | 7.97±0.31 | 7.916±0.18 | 14.86±0.68 | 5.829±0.39 |
| 3 | 3 | 11.62±0.39 | 11.57±0.56 | 18.87±1.1 | 8.594±1.13 |
| 4 | 4 | 15.74±0.48 | 15.69±0.48 | 22.74±0.79 | 12.72±0.48 |
| 5 | 5 | 20.75±0.33 | 20.7±0.24 | 25.61±0.65 | 17.62±0.71 |
| 6 | 6 | 25.19±0.41 | 25.14±0.5 | 28.48±1.13 | 22.79±1.04 |
| 7 | 7 | 29.26±0.47 | 29.21±0.63 | 32.34±0.96 | 26.28±0.87 |
| 8 | 8 | 32.75±0.24 | 32.7±0.41 | 35.52±1.1 | 31.08±1.02 |
| 9 | 9 | 37.82±0.65 | 37.76±0.72 | 39.38±1.01 | 34.68±1.22 |
| 10 | 10 | 43.5±0.56 | 43.45±0.74 | 45.54±0.41 | 41.42±0.18 |
| 11 | 24 | 96.62±0.39 | 96.57±0.27 | 95.79±0.72 | 94.59±0.79 |

All the values were expressed as mean S.D., n*=3

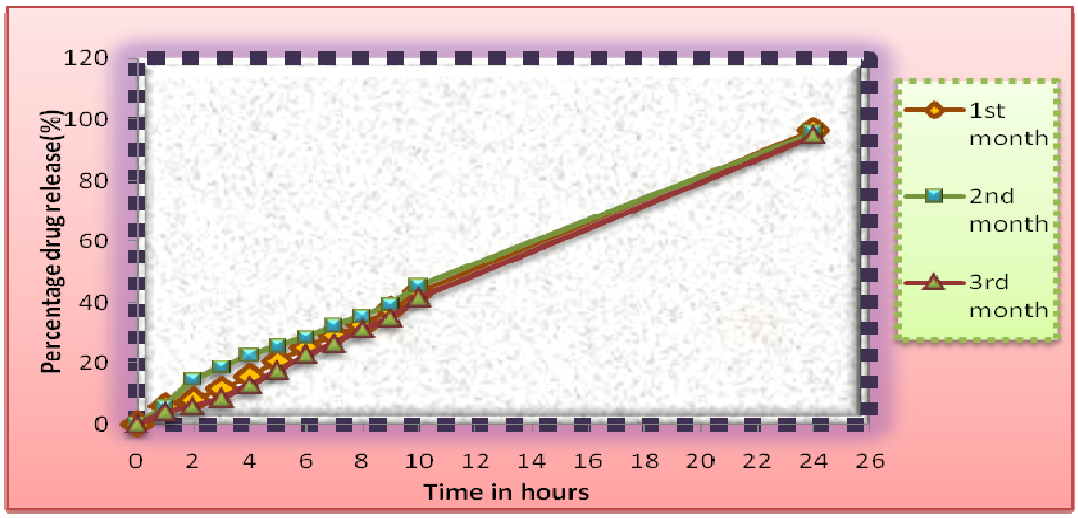


**Fig. 9.45:** Percentage *in-vitro* drug release of selected formulation EN5 after stability studies at refrigerator temperature

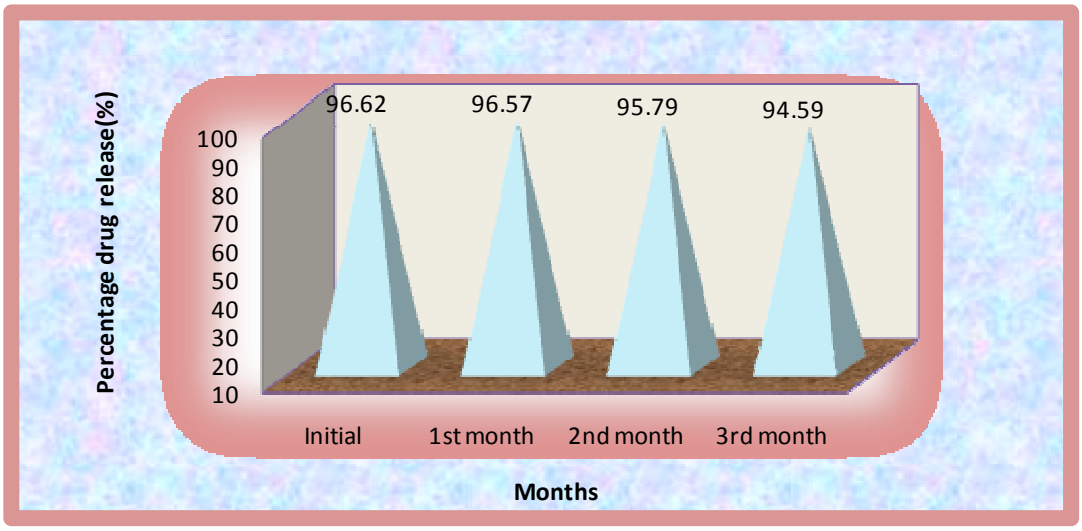


**Fig. 9.46:** Comparison of in vitro drug release before and after stability studies at refrigerator temperature for optimized formulation EN5

**Table 9.20:** Stability studies of optimized formulation EN5 at room temperature

| Characteristic | Initials | 1st Month | 2nd Month | 3th Month |
|---|---|---|---|---|
| **Drug retained (%)** | 92.10 | 91.13 | 90.82 | 90.59 |
| **In vitro drug release at 24 hour* (%)** | 96.62±0.39 | 96.42±0.27 | 95.63±0.98 | 94.43±0.55 |
| **Test for sterility** | Sterile | Sterile | Sterile | Sterile |

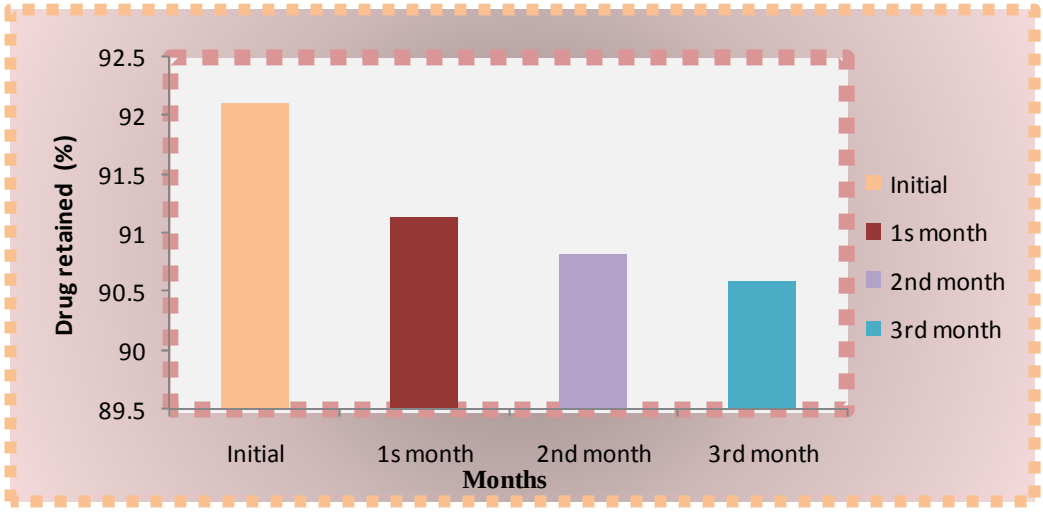


**Fig. 9.47:** Comparison of drug retained (%) before and after stability studies at room temperature for optimized formulation EN5

**Table 9.21:** Percentage *in-vitro* drug release of selected formulation EN5 after stability studies at room temperature

| S. No | Time hours | Room temperature | | | |
|---|---|---|---|---|---|
| | | Initial* | 1st month* | 2nd month* | 3rd month* |
| 1 | 1 | 5.88±0.48 | 5.776±0.47 | 5.881±0.18 | 3.95±0.45 |
| 2 | 2 | 7.97±0.31 | 7.759±0.24 | 14.7±0.87 | 5.672±0.65 |
| 3 | 3 | 11.62±0.39 | 11.41±0.83 | 18.72±0.89 | 8.438±0.95 |
| 4 | 4 | 15.74±0.48 | 15.53±0.24 | 22.58±1.02 | 12.56±0.24 |
| 5 | 5 | 20.75±0.33 | 20.54±0.18 | 25.45±0.86 | 17.57±0.94 |
| 6 | 6 | 25.19±0.41 | 24.98±0.24 | 28.32±1.34 | 22.63±1.02 |
| 7 | 7 | 29.26±0.47 | 29.05±0.45 | 32.18±0.71 | 26.13±0.68 |
| 8 | 8 | 32.75±0.24 | 32.55±0.31 | 25.36±1.34 | 30.93±1.07 |
| 9 | 9 | 37.82±0.65 | 37.61±0.45 | 39.22±0.86 | 34.53±1.42 |
| 10 | 10 | 43.5±0.56 | 43.29±0.71 | 45.38±0.16 | 41.26±0.45 |
| 11 | 24 | 96.62±0.39 | 96.42±0.27 | 95.63±0.98 | 94.43±0.55 |

All the values were expressed as mean S.D., n*=3

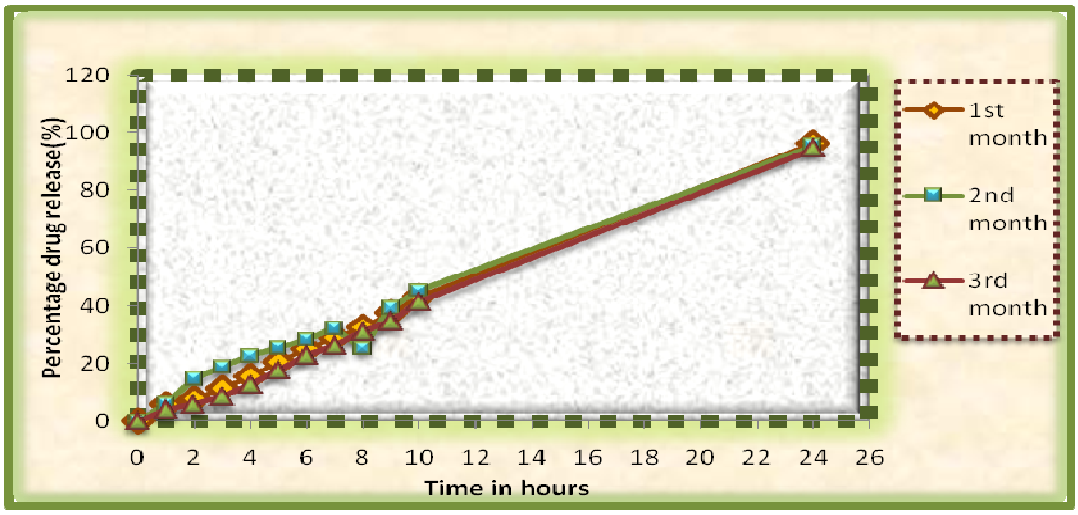


**Fig. 9.48:** Percentage *in-vitro* drug release of selected formulation EN5 after stability studies at room temperature

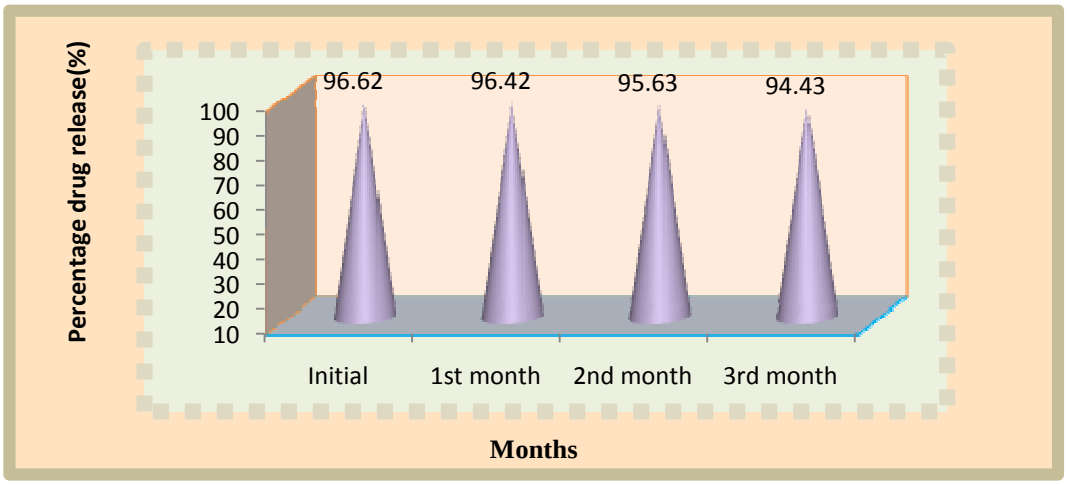


**Fig. 9.49:** Comparison of in vitro drug release before and after stability studies at room temperature for optimized formulation EN5

**Table 9.22:** Stability studies of optimized formulation EN5 at elevated temperature.

| Characteristic | Initials | 1st Month | 2nd Month | 3th Month |
|---|---|---|---|---|
| **Drug retained (%)** | 92.10 | 91.28 | 90.18 | 89.25 |
| **In vitro drug release at 24 hour* (%)** | 96.62±0.39 | 95.68±0.33 | 94.8±1.01 | 93.7±0.63 |
| **Sterility testing** | Sterile | Sterile | Sterile | Sterile |

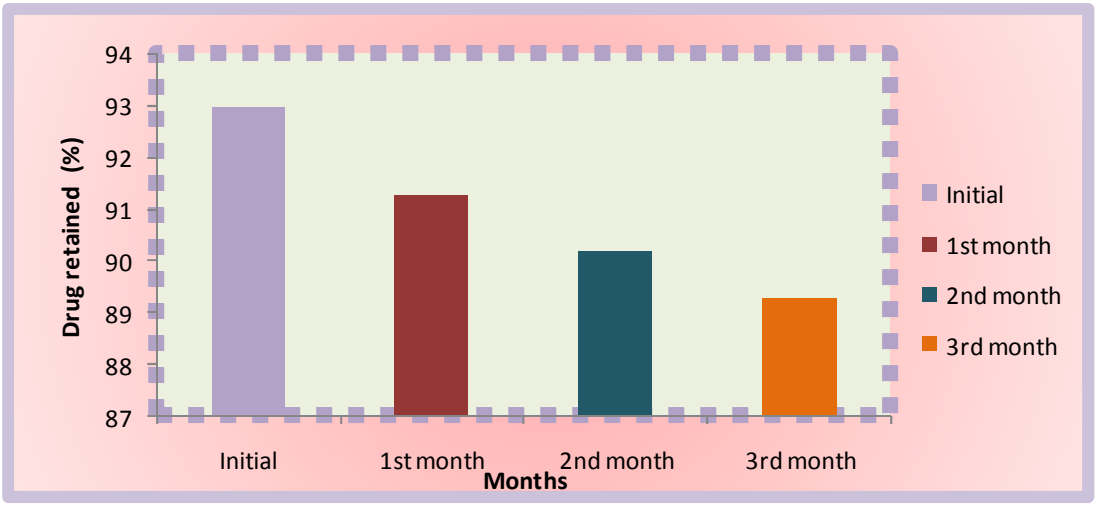


**Fig. 9.50:** Comparison of drug retained (%) before and after stability studies at elevated temperature for optimized formulation EN5

**Table 9.23:** Percentage *in-vitro* drug release of selected formulation EN5 after stability studies at elevated temperature

| S. No | Time hours | Elevated temperature | | | |
|---|---|---|---|---|---|
| | | Initial* | 1st month* | 2nd month* | 3rd month* |
| 1 | 1 | 5.88±0.48 | 5.046±0.55 | 5.046±0.24 | 3.115±0.41 |
| 2 | 2 | 7.97±0.31 | 6.977±0.24 | 13.92±0.87 | 4.889±0.65 |
| 3 | 3 | 11.62±0.39 | 10.63±0.83 | 17.83±0.89 | 7.759±0.86 |
| 4 | 4 | 15.74±0.48 | 14.75±0.09 | 21.8±1.02 | 11.88±0.16 |
| 5 | 5 | 20.75±0.33 | 19.81±0.24 | 24.67±0.86 | 16.68±0.94 |
| 6 | 6 | 25.19±0.41 | 24.25±0.16 | 27.48±1.33 | 21.85±1.02 |
| 7 | 7 | 29.26±0.47 | 28.27±0.45 | 31.5±1.04 | 25.34±0.68 |
| 8 | 8 | 32.75±0.24 | 31.36±0.31 | 34.63±1.33 | 30.2±0.98 |
| 9 | 9 | 37.82±0.65 | 36.88±0.5 | 38.39±0.77 | 33.75±1.42 |
| 10 | 10 | 43.5±0.56 | 42.51±0.71 | 44.6±0.16 | 40.48±0.33 |
| 11 | 24 | 96.62±0.39 | 95.68±0.33 | 94.8±1.01 | 93.7±0.63 |

All the values were expressed as mean S.D., n*=3

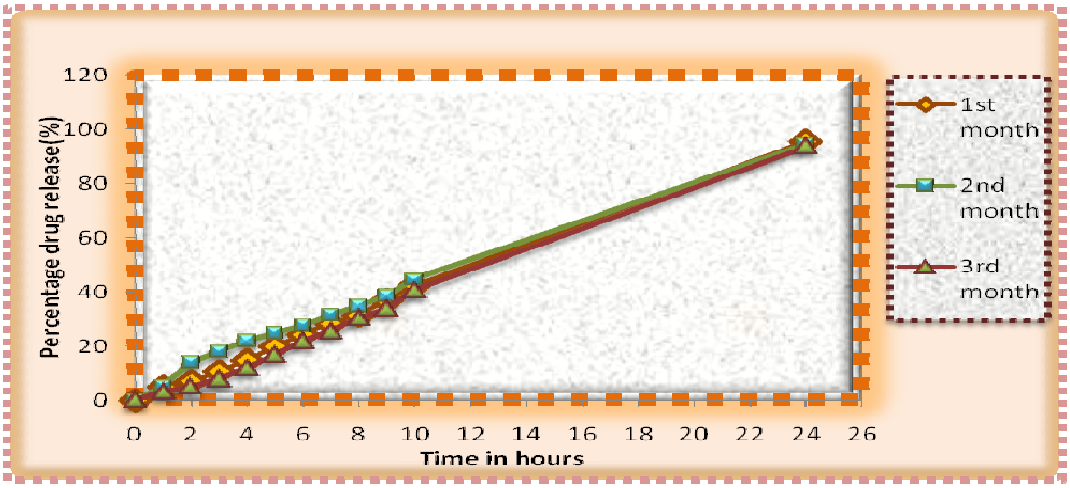


**Fig. 9.51:** Percentage *in-vitro* drug release of selected formulation EN5 after stability studies at elevated temperature

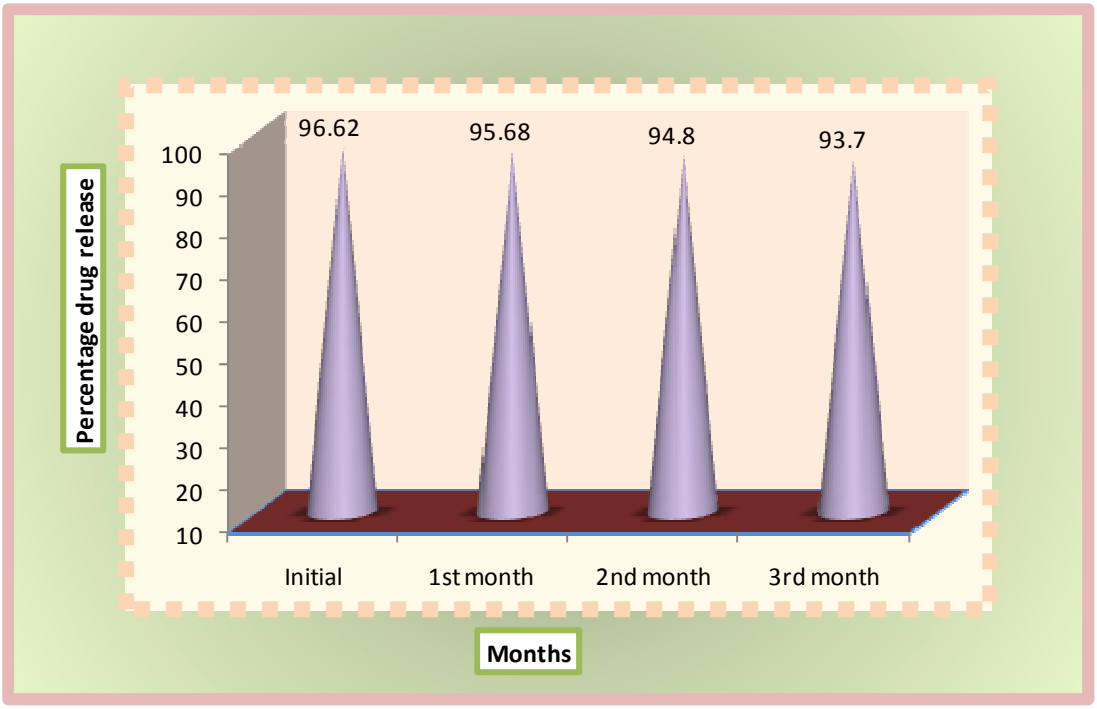


**Fig. 9.52:** Comparison of in vitro drug release before and after stability studies at elevated temperature for optimized formulation EN5

The studies reveal that, there were no much significant changes in percentage drug retained after storage for three months at 4ºC, 25ºC and 40ºC. It was observed that the leakage from the vesicle was least at 4ºC followed by 25ºC and 40ºC. *In- vitro* drug release studies revealed that the formulation stored at 4ºC showed 94.59% release, the one stored at 25ºC showed 94.43% release and 40ºC batch showed 93.7% release after 24 h.

These result indicate that the drug release from the formulation stored at 4ºC was highest followed by formulation stored at 25ºC and 40ºC. This may be attributed to phase transition of surfactants and lipid causing vesicle leakage at higher temperature during storage. Hence it was concluded from the obtained data, that the optimum storage condition for niosomes found to be 4ºC.

# Summary And Conclusion

# 10. SUMMARY AND CONCLUSION

The present study was aimed to develop a novel niosomal drug delivery system for the treatment of different types of cancer. Nine batches of niosomes (EN1, EN2, EN3, EN4, EN5, EN6, EN7, EN8, and EN10) were prepared by using various surfactants cholesterol and drug with $3^2$ factorial design by Reverse phase evaporation method.

Preformulation study was carried out for crude drug. The initial part of work was started from the identification of drug. Identification of drug was determined by melting point and solubility.

The niosomes recovery (%) of the formulated niosomes was in the optimum range, which indicates a better niosomal recovery. The entrapment efficiency was more than fifty percent with all niosomal preparation. The maximum drug entrapment was found only in formulation EN5. Entrapment efficiency was found to be a function of equimolar concentration of X1 (polysorbate 80) and X2 (cholesterol). The drug loading efficiency of all niosomes showed acceptable range.

The shape of niosomes was found to be smooth, spherical and bilayered by SEM analysis. Smooth surface reveals complete removal of solvent from the formulated niosomes and this was the indication of good quality.

The particle size data showed that niosomes produced were of nanosize and had low polydispersity index which indicates relatively narrow particle size distribution for all formulations.

Zeta potential of all formulated noisome was found to posses the optimum surface free energy, which indicates moderate stability with no agglomeration.

The compatibility studies by FTIR and DSC analysis of niosomes suggest that the formulation components polysorbate 80 and cholesterol and the drug Etoposide do not interact to form any additional chemical entity but remain as a mixture. Therefore, it could indicate that there was no incompatibility between drug and excipients. The similarity in peaks indicates there is no incompatibility between drug and the surfactants used for formulation.

From *in-vitro* release studies it was concluded that formulation EN5 containing surfactant and cholesterol in equimolar ratio of 1:1 found to be best formulation among other formulations, which showing the most desired drug release This was followed by a steady drug release pattern, which approximated zero order release. It will be considered as optimized formulation.

The optimized formulation EN5 was subjected to sterilization, sterility test and *in-vivo* drug targeting studies and stability studies. The formulation was found to be sterile.

The *in-vivo* tissue distribution results revealed that, the drug loaded niosomes showed preferential drug targeting to liver followed by spleen, lungs, kidney and brain. It was also revealed that, as compared to pure drug, higher concentration of drug was targeted to the organs like liver and lungs after administering the dose in the form of niosomes.

The stability studies revealed that the leakage from the vesicle was least at 4ºC followed by 25ºC and 40ºC. The studies reveal that, there were no much significant

changes in percentage drug retained after storage for three months at 4ºC, 25ºC and 40ºC. The drug release from the formulation stored at 4ºC was highest followed by formulation stored at 25º C and 40ºC. Hence it was concluded from the obtained data, that the optimum storage condition for niosomes found to be 4ºC.

Out of the nine formulations, it appears that **Formulation EN5** has the maximum potential in providing niosomal drug delivery system. This formulation was considered as best formulation for targeting different type of cancer in order to the information obtained from *in-vitro* and *in-vivo* studies.

# FUTURE PROSPECTS

# 11. FUTURE PROSPECTS

In the present work the Novel niosomal delivery system of Etoposide were prepared by using polysorbate 80 and cholesterol with $3^2$ factorial design by Reverse phase evaporation method. In this investigation, the important parameters like physico chemical characterization, *in-vitro* evaluation, sterility studies and *in vivo* tissue distribution studies of novel niosomal delivery system of Etoposide were done. Further detailed investigation and elaborate in-vivo targeting studies using human volunteers need to be carried out to establish efficacy of this formulation.

In order to have a comparative evaluation of *in-vivo* performance such as pharmacokinetic analysis will be performed on plasma concentration time profiles of this formulation after i.v. route of administration, since plasma profile is an important aspect in understanding biological action of a drug. *In-vitro in-vivo* correlation studies to be performed in future.

# Bibliography

## 12.BIBLIOGRAPHY


1. Abdul Hasan Sathali A. and Rajalakshmi G. Evaluation of Transdermal Targeted Niosomal Drug Delivery of Terbinafine Hydrochloride, *International Journal of Pharmaceutical Technology and Research,* **2010**, 2(3), 2081-2089.
2. Ajay B. Solanki, Jolly R. Parikh, Rajesh H. Parikh and Mrunali R. Patel. Evaluation of Different Compositions of Niosomes to Optimize Aceclofenac Transdermal Delivery, *Asian Journal of Pharmaceutical Sciences,* **2010**, 5(3), 87-95.
3. Anand Kumar Y., Srinivas S., Hemanth A. and Anitha M. Preparation and Evaluation of Niosomes Containing Aceclofenac, *Digest Journal of Nanomaterials and Biostructures,* **2010**, 5(1), 249-254.
4. Anonymous, http://www.en.wikipedia.org/wiki/Etoposide.
5. Anonymous, http:// www.google.com, drug bank
6. Anonymous, http://www.pharmainfo.net
7. Anonymous, http://www.rxlist. Etoposide.
8. Anonymous, http://www.cancer.org.
9. Anonymous. Indian Pharmacopoeia. 5 th edn., Government of India, Ministry of Health and Family Welfare, The Indian Pharmacopoeial commission, Ghaziabad, **2007**, 496-500.
10. Anonymous, The United States Pharmacopoeia XXXII, NF XXVII. The United States Pharmacopoeial convention, Rockville, MD, USA. Volume I, **2009**, 1101, 1192.

11. Anupriya kapoor, R.Gahoi and D.Kumar. In-Vitro Drug Release Profile of Acyclovir from Niosomes formed with Different Sorbitan Esters, *Asian Journal of Pharmacy and Life Science*, **2011**, 1(1), 64-70.
12. Azmin M.N., Florence A. T., Stuart J.F.B., Vanlerberghes G. and Whittaker J. S. The Effect of Niosomes and Polysorbate 80 on the Metabolism and Excretion of Methotrexate in the Mouse, *Journal of Microencapsulation*, **1986**, 3(2), 95-100.
13. Balasubramanian A, Dasaratha D.M and Vangala A.K. Formulation and in-vitro evaluation of niosomal encapsulated Daunorubicin hydrochloride, *Indian Drugs*, **2002**, 39(1), 27-31.
14. Banker S.G. and Rhodes C.T. Modern Pharmaceutics, 4th edn., Marcel Dekker, Inc, New York, **2008**, 529-576.
15. Brahmankar D.M. and Jaiswal S.B. Biopharmaceutis and Pharmacokinetics: A treatise. 2nd edn., Vallabh Prakashan, New Delhi, **2006**, 64-65, 335-357.
16. Chawda Himmat Singh, Jain C. P. and Bairwa Narendra Kumar. Formulation, Characterization, Stability and In vitro Evaluation of Nimesulide Niosomes, *Pharmacophore*, **2011**, 2 (3), 168-185.
17. David G. Rhodes and Almira I. Blazek-Welsh. SEM Imaging Predicts Quality of Niosomes from Maltodextrin-Based Proniosomes, *Pharmaceutical Research*, **2001**, 18(5), 656-661.
18. Doijad R.C, Mani F.V, Swati S and Rony M. J. Niosomal drug delivery of cisplatin- Development and characterization, *Indian Drugs*, **2008,** 45(9), 713-718.

19. Dubey subodh, Jain Amit, Mehtha S.C., Gupta Pavan, Jain sandeep and Sahu Jagdish. Niosome: An Ultimate Drug Carrier, *Drug Invention Today*, **2010**, 2(1), 72-77.
20. Farhan J. Ahmad, Purnima Negi, Dabeer Ahmad, Gaurav K. Jain and Gyanendra Singh. Development of a Novel Formulation for Transdermal Delivery of an Antidepressant Drug, *International Journal of Pharmaceutical Sciences and Research*, **2011,** 2(7), 1766-1771.
21. Gayathri Devi S. Vekatesh and N. Udupa. Niosomal Sumatriptan succinate for nasal administration, *Indian Journal of Pharmaceutical Sciences,* **2000**, 479-481.
22. Ghada Abdelbary, and Nashwa El-gendy. Niosome-Encapsulated Gentamicin for Ophthalmic Controlled Delivery, *AAPS Pharmaceutical Science and Technology,* **2008**, 9(3), 740-747.
23. Goodman and Gillman's. The Pharmacological Basis of Therapeutics Mc Graw-Hill Medical Publishing Division, New York, **2008**, 853-908.
24. Gupta Manish and Sharma Vimukutha. Targeted Drug Delivery System: A Review, *Research journal of chemical sciences,* **2011**, 1(2), 135-138.
25. Gyanendra Singh, Hemant K Raghuvanshi, Anubhav Anand, Rakesh Pundir and Harinath Dwivedi. Targeted Delivery of Rifampicin by niosomal drug delivery system, *Journal of Pharmacy Research*, **2010**, 3(5), 1152-1154.
26. Heinz Lullman, Klaus Mohr, Albert Ziegler and Detlef Bieger. Color Atlas Of Pharmacology, 2nd edn., Georg Thieme Verlag, New York, **2000**, 296-299.

27. Ismail Mouzam, Dehghan M.H.G. and Shaikh Samina M. Development and Characterization of Salmeterol Xinafoate Niosomes for Nasal Delivery, *Indian Journal of Pharmaceutical Education and Research*, **2011**, 45(2), 121-127.

28. Jaydeep D Yadav, Priyanka R., Kulkarni A Vaidya and Gurubas T Shelke. Niosomes: A Review, *Journal of Pharmacy Research*, **2011,** 4(3), 632-636.

29. Jigar Vyas, Puja Vyas, Dhaval Raval, and Paresh Paghdar. Development of Topical Niosomal Gel of Benzoyl Peroxide, *International Scholarly Research Network*, **2011,** 1-6.

30. Kandasamy Ruckmani and Veintramuthu Sankar. Formulation and optimization of Zidovudine niosomes, *AAPS Pharmaceutical Science and Technology*, **2010,** 11(3), 1119-1127.

31. Khar R.K. and Vyas S.P. Controlled drug delivery concepts and advances, Vallabh Prakashn Publishers, Delhi, **2008**, 511-534.

32. Kenneth Hande, Mark Messenger, Judy Wagner, Mary Krozely and Sanjeev Kaul. Inter- and Intrapatient Variability in Etoposide Kinetics with Oral and Intravenous Drug Administration, *Clinical Cancer Research*, **1999**, 5, 2742–2747.

33. Kumar Abhinav, Pal jogender lal, Jaiswal Amit and Sing Vishwabhan. Review on niosomes as novel drug delivery system, *International research journal of pharmacy*, **2011**, 2(5), 61-65.

34. Lakshmi P.K. and Shyamala Bhaskaran. Comparative Evaluation of Niosome Formulations Prepared by Different Techniques, *Acta Pharmaceutica Sciencia*, **2009**, 51, 27-32.

35. Lakshmi P.K. and Shyamala Bhaskaran. Phase II Study of Topical Niosomal Urea Gel - An Adjuvant in the Treatment of Psoriasis, *International Journal of Pharmaceutical Sciences Review and Research*, **2011**, 7(1), 1-7.

36. Magharla Dasaratha Dhanaraju, Nandhakumar Sathyamoorthy,Vankayalu Devendiran Sundar, Suresh C. Preparation of poly (epsilon-caprolactone) microspheres containing etoposide by solvent evaporation method, *Asian Journal of Pharmaceutical Sciences*, **2010**, 5 (3), 114-122.

37. Manavalan R. and Ramasamy S. Physical Pharmaceutics, Vignesh Publisher, Chennai, **2004,** 288-299.

38. Manivannan Rangasamy, Balasubramaniam Ayyasamy and Senthilkumar Raju. Formulation and *in vitro* Evaluation of Niosome Encapsulated Acyclovir, *Journal of Pharmacy Research*, **2008**, 1(2), 163-166.

39. Meenakshi Bharkatiya, Rajesh Kumar Nema and Maheep Bhatnagara. Formulation and Evaluation of Metoprolol Tartrate Entrapped Niosomes**,** *International Journal of Chemical Sciences*, **2010**, 8(4), 2055-2062.

40. Pandey Shivanand. Development and Characterization of Cefpodoxime Proxetil Niosomes, *International Journal of Pharma World Research*, **2010,** 1(3), 1-11.

41. Pavala Rani N., Suriyaprakash T.N.K. and Senthamarai R. Formulation and Evaluation of Rifampicin and Gatifloxacin Niosomes on Logarithmic-Phase Cultures of Mycobacterium Tuberculosis, *International Journal of Pharma and Bio Sciences*, **2010,** 1(4), 379-387.

42. Popli H and Mini S.Nair. Niosomal delivery of Tenoxicam, *Indian Journal of Pharmaceutical Sciences*, **1996,** 163-166.

43. Pratap S. Jadon, Virendra Gajbhiye, Rajesh S. Jadon, Kavita R. Gajbhiye and Narayanan Ganesh. Enhanced Oral Bioavailability of Griseofulvin via Niosomes, *AAPS Pharmaceutical Science and Technology*, **2009**, 10(4), 1186-1192.

44. Rajesh Z. Mujoriya, Ramesh Babu Bodla, Kishore Dhamanle, Devendra Singh and Logesh Patle. Niosomal Drug Delivery System: A Magic Bullet, *Journal of Applied Pharmaceutical Science*, **2011**, 1(9), 20-23.

45. Raju Jukanthi, Deepthi Annakula, Madhukar Rao Errabelli, Suresh Bandari and Prabakar Reddy Verareddy. Provesicular Drug Delivery System: An Overview and Appraisal, *Scholars Research Library*, **2010**, 2(4), 135-146.

46. Ravi Kumar, Shivji Kumar, Shyam Shankar Jha and Amit Kumar Jha. Vesicular System – Carrrier For Drug Delivery, *Pelegia Research Library*, **2011**, 2(4), 192-202.

47. Raymond C Rowe, Paul J Sheskey and Paul J Weller. Hand book of pharmaceutical excipients, Pharmaceutical press, London, **2006**, 182-184, 580-584.

48. Robinson J.R. and Lee V.H.L. Controlled drug delivery fundamentals and applications, 2nd edn., Informa Health care, Inc., New York, **2009**, 462-463.

49. Roopa Karki, Mamatha G.C., Subramanya G. and Udupa N. Preparation, Characterization and Tissue Disposition of Niosomes Containing Isoniazid, *Rasayan Journal of Chemistry*, **2008**, 1(2), 224-227.

50. Sambhakar S., Singh B., Paliwal S.K. and Mishra P.R. Niosomes as a Potential Carrier for Controlled Release of Cefuroxime Axetil, *Asian Journal of Biochemical and Pharmaceutical Research*, **2011**, 1(1), 126-136.

51. Sanaa A. El-Gizawy, Ismail A. Attia, Medhat A. Fouda and Ahmed M. Donia. Influence of a Niosomal Formulation on the Oral Bioavailability of Acyclovir in Rabbits, *AAPS Pharmaceutical Science and Technology*, **2007**, 8(4), 1-7.

52. Sandeep Kumar Sharma, Meenakshi Chauhan and Narayanapillay Anilkumar. Span-60 Niosomal Oral Suspension of Fluconazole: Formulation and *In-Vitro* Evaluation, *Journal of Pharmaceutical Research and Health Care*, **2009**, 1(2), 142-156.

53. Shobha Rani R.H. Text book of Industrial Pharmacy drug delivery systems, and cosmetics and Herbal drug technology, Orient Longman Private Ltd, Chennai, **2008,** 1-3, 98-125.

54. Skoog D.A, Holler F. and Nieman S. Principles of instrumental analysis. 5th edn., Thomson Asia Pvt. Limited, Singapore, **1996**, 410-413.

55. Stanley R. Hamilton and Laure A. Aaltonen. Pathology and Genetics of Tumours of the Digestive System, IARC Press, Lyon France, **2000**, 9, 20, 57, 69,157.

56. Surender Verma, S.K. Singh, Navneet Syan, Pooja Mathur and Vinay Valecha. Nanoparticle vesicular system: A versatile tool for drug delivery, *Journal of Chemical and Pharmaceutical Research*, **2010**, 2(2), 496-509.

57. Sweetman S.C., Martindale The Complete drug reference, 36th edn., The Pharmaceutical press, London, **2009,** 718.

58. Varaporn Buraphacheep Junyaprasert, Veerawat Teeranachaideekul and Tasaneeya Supaperm. Effect of Charged and Non-ionic Membrane Additives

on Physicochemical Properties and Stability of Niosomes, *AAPS Pharmaceutical Science and Technology*, **2008**, 9(3), 851-859.

59. Vedha Hari B.N. and Samyuktha Rani B. Niosomal Formulation of Orlistat: Formulation and In-Vitro Evaluation, *International Journal of Drug Development & Research*, **2011**, 3(3), 300-311.
60. Vyas Jigar, Vyas Puja, and Sawant Krutika. Formulation and Evaluation of Topical Niosomal Gel of Erythromycin, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2011**, 3(1), 123-126.
61. Vyas SP, Khar R.k. Targeted and Controlled drug delivery, Novel carrier systems, 1st edn, CBS publishers, New Delhi, **2002**, 39, 249, 331.
62. Yan-Zhuo Zhang, Jin-Ming Li, Jun-Gang Ren, and Yun-Zhi Qu. Preparation of Stealthy Etoposide Proliposomes and the Pharmacokinetics in Rabbits, *Journal of Chinese Pharmaceutical Sciences*, **2008**, 17, 303–308.